



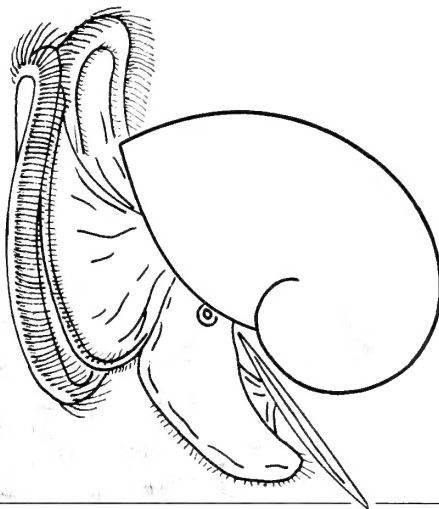








# THE VELIGER



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Volume 45

*January 2, 2002 to October 1, 2002*



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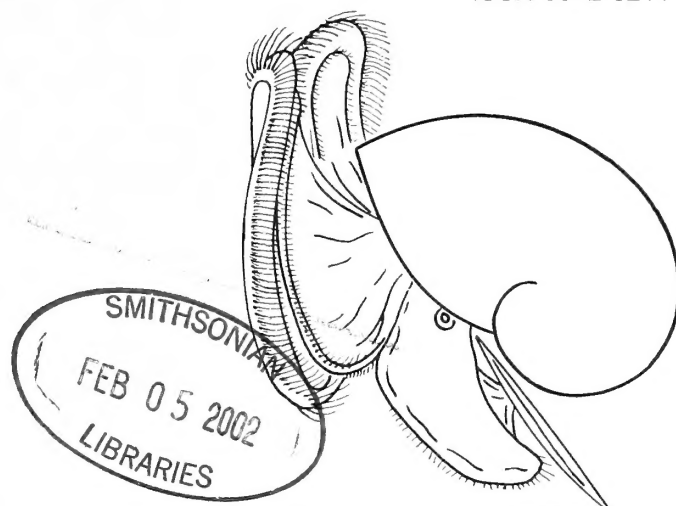
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## THE VELIGER

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*The Veliger* is an international, peer-reviewed scientific quarterly published by the California Malacozoological Society, a non-profit educational organization. *The Veliger* is open to original papers pertaining to any problem connected with mollusks. Manuscripts are considered on the understanding that their contents have not appeared, or will not appear, elsewhere in substantially the same or abbreviated form. Holotypes of new species must be deposited in a recognized public museum, with catalogue numbers provided. Even for non-taxonomic papers, placement of voucher specimens in a museum is strongly encouraged and may be required.

Very short papers, generally not over 750 words, will be published in a "Notes, Information & News" column; in this column will also appear notices of meetings and other items of interest to our members and subscribers.

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## Northeastern Pacific Sacoglossan Opisthobranchs: Natural History Review, Bibliography, and Prospectus

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**Abstract.** The species richness and geographic ranges of the sacoglossan (synonym: ascoglossan) opisthobranch fauna have been well characterized for northeastern Pacific shores, particularly in the Californian province, but the natural history and ecology of these gastropods have been comparatively less well studied. Over half of the described sacoglossan genera and approximately two-thirds of the families are represented on northeastern Pacific shores. At least 25 species of sacoglossans occur: eighteen species are known stenophagous herbivores, and one feeds on opisthobranch eggs. Eight species occur on cold-temperate northeastern Pacific shores, 19 species inhabit the Gulf of California and warm-temperate to tropical Pacific shores, and four species occur in the Aleutian, Oregonian, Californian, and Panamic provinces. Five of the species have been studied appreciably more than the others: *Elysia hedgpethi* (Marcus, 1961), *Alderia modesta* (Lovén, 1844), *Placida dendritica* (Alder & Hancock, 1843), *Aplysiopsis enteromorphae* (Cockerell & Eliot, 1905), and *Stiliger fuscovittatus* Lance, 1962. The paucity of study on other species is not necessarily due to low abundance. This natural history review of the regional sacoglossan fauna synthesizes the scattered literature about the stenophagous gastropods and highlights the major gaps that malacologists should seek to fill in the study of this highly specialized order. Future research should focus more on the autecology, population ecology, and community ecology of sacoglossans. Recent advances in isotope analysis, fluorometry, larval culturing, and molecular techniques provide challenging opportunities to enhance our understanding of sacoglossan biology.

### INTRODUCTION

Sacoglossan (synonym: ascoglossan) opisthobranchs are small sea slugs that are suctorial feeders on marine algae, seagrass, diatoms, and opisthobranch eggs (Williams & Walker, 1999). Our knowledge of the northeastern Pacific sacoglossan fauna is quite recent, with a rapid proliferation in species recognized since 1960 (Figure 1). Many of the northeastern Pacific species that are broadly distributed were first described in other parts of the world (Figure 1) and then subsequently recorded as present on northeastern Pacific shores. The rate of species discovery is still high (e.g., Farmer, 1996; Lance, 1998; J. Goddard, personal communication, 2000; Valdés & Camacho-García, 2000). Whether such finds are due to (1) locating easily overlooked species (i.e., problem of omission), (2) previous lack of study (Lee & Foster, 1985), or (3) establishment of introduced species is not entirely clear. Most of the recent discoveries have been in the Panamic province.

Beeman & Williams (1980:309) astutely noted: "Studies of California opisthobranchs to date have been mainly taxonomic and distributional in nature." In addition, not only is classification within the order Sacoglossa highly unstable (Roller, 1970a; Marcus, 1982; Gascoigne, 1985; Jensen, 1996; Burn, 1998; Williams & Walker, 1999; Mikkelsen, 1998) but also the proper name of the order

and several species are controversial (Keen, 1973; Jensen, 1991; K. B. Clark, personal communication, 1986; Marshall & Willan, 1999). There remains a continued division in usage of the terms Ascoglossa vs. Sacoglossa; the controversy has been exacerbated by numerous prominent authors switching terms between papers and, in at least one case, within papers. After extensive discussions with taxonomists, I now change from my past use of Ascoglossa to Sacoglossa. Despite the taxonomic and nomenclatural issues, however, in the last two decades, our knowledge of northeastern Pacific species has substantially matured with studies on the ecology, ecophysiology, and reproductive biology of sacoglossan slugs.

The order has recently been reviewed from a number of different perspectives: feeding ecology (Williams & Walker, 1999), kleptoplasty (Clark et al., 1990; Clark, 1992; Williams & Walker, 1999), population ecology (Clark & DeFreese, 1987), and taxonomy (Jensen 1996, 1997; Mikkelsen, 1998). For many geographic regions, there are admirable syntheses of the sacoglossan fauna, their biology and ecology. The present paper (1) synthesizes the existing, broadly scattered details, (2) highlights an unpublished M.A. thesis (Case, 1972) on *Stiliger fuscovittatus* cited only once (by Behrens, 1980) in the past three decades, and (3) evaluates our present understanding of the northeastern Pacific sacoglossan assemblage. Because of these objectives, some of the topics in this



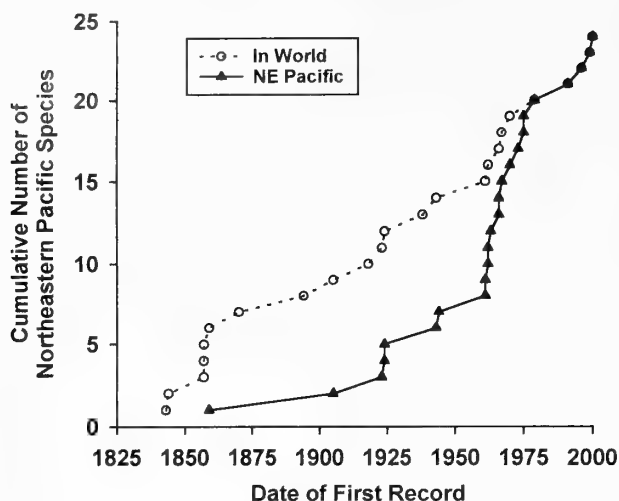


Figure 1. Temporal pattern of species discovery and/or description for northeastern Pacific sacoglossans. Open circles represent first records, for other parts of the world, of species now known from northeastern Pacific shores; closed triangles represent first records of northeastern Pacific species on these shores. Data based on Cockerell & Eliot, 1905; Agersborg, 1923; MacFarland, 1924, 1966; O'Donoghue, 1924; Pilsbry & Olsson, 1943; Sowell, 1949; Hand, 1955; Marcus, 1961; Keen & Smith, 1961; Lance, 1962; Marcus & Marcus, 1967, 1970a, b; Keen, 1971; Sphon, 1971; Sphon & Mulliner, 1972; Ferreira & Bertsch, 1975; Oakes, 1979; Behrens, 1991a; Valdés & Camacho-García, 2000; J. Goddard, personal communication, 2000.

review (e.g., phenology) are necessarily based on personal communications, observations, or unpublished data by professional colleagues; quantitative descriptions and experimental underpinnings will be (or should be) published in due course. Finally, this review does not attempt to clarify the taxonomic identities of the undescribed species or to resolve issues of problematic species. The primary objective is to provide a cohesive understanding of past work and to provide a focused prospectus for future sacoglossan research.

### TAXONOMIC RICHNESS

The number of species, termed "species richness," varies depending on the author and the specific range considered. McDonald (1975) listed seven species for the central California coast, Ricketts et al. (1985:562) mentioned "seven or eight species on our coast" and provided some early references for the Pacific coast, and Beeman & Williams (1980) provided excellent descriptions of two species and briefly mentioned four other species on Californian shores. Farmer (1980) provided comprehensive summaries of nine species for the northeastern Pacific, incorporating the Gulf of California species, and Keen (1971) summarized the species on tropical eastern Pacific shores. Behrens (1991a, b) illustrated and briefly described 12 species and mentioned one other. If these records are

merged, there are at least 25 species (20 described, five undescribed) on northeastern Pacific shores between Alaska and Baja California, including the Gulf of California (Table 1). With increased study of low latitude northeastern Pacific shores (Mexico to northern Ecuador), more species will undoubtedly be discovered.

In terms of higher-level taxonomic diversity, Jensen (1996) listed nine families, 23 genera, and approximately 200 species worldwide in the order Sacoglossa. Based on Jensen's (1996) classification system, there are an estimated 66.7% of the families represented, 60.9% of the genera, and 12.5% of the species in the northeastern Pacific region. Depending on the specific boundaries recognized between biogeographical provinces (Vermeij, 1978; Hartman & Zahary, 1983), the species richness varies (Table 1). There are substantially more sacoglossans known from the Panamic Province (Gulf of California and northeastern Pacific, from Bahía Magdalena south) than from the northern provinces.

### GEOGRAPHIC RANGES

Our knowledge of the geographic ranges of these species (Tables 2, 3) is incomplete, owing to gaps in sampling and, in some cases, a lack of sampling outside known ranges. Bertsch (1973:51) noted: "The ranges probably reflect the concentration of study in a few areas as much as the actual ranges of the species." The vast majority of reports on northeastern Pacific sacoglossans are listed as "range extensions," implicitly indicating increases in known ranges. Such records should not be considered "range extensions" in the strictest sense because there is no evidence that species are modifying their ranges but rather our knowledge of the ranges is changing (Clark, 1997); malacological terminology is presently inconsistent with other fields (e.g., population ecology and invasion biology) and thus subject to confusion.

All of the species found on cold-temperate and boreal shores (Oregonian and Aleutian Provinces) are also found in the Californian Province; four of these species also occur in the Gulf of California (Panamic Province) (Table 1). With the possible exception of the seemingly uncommon *Aplysiopsis oliviae*, *Placida* sp. 1, and *Olea hansineensis*, most of the other Pacific coast species are widely distributed in the northeastern Pacific. The southern extent of the ranges of species found in the Gulf of California is generally not well known; this undoubtedly reflects the paucity of opisthobranch studies on low-latitude, northeastern Pacific shores.

Three species have curious and perhaps questionable ranges: *Ercolania fuscata*, *E. boodleae*, and *Stiliger fuscovittatus*. (1) *Ercolania fuscata* occurs on northwestern Atlantic shores (Clark, 1975), the tip of South America, possibly southeastern Australia (Thompson, 1973; but see Jensen & Clark, 1983), and the Gulf of California; as Ferreira & Bertsch (1975) emphasized, this distribution

Table 1

Sacoglossan opisthobranchs on northeastern Pacific shores. Classification based on Jensen (1996). *Cylindrobulla californica* Hamatani, 1971, is not included because Jensen (1996) excluded the family Cylindrobullidae from the order Sacoglossa (but see Mikkelsen, 1998). Provinces based on Vermeij (1978) and Hartman & Zahary (1983).

Species	Aleutian Province	Oregonian Province	Californian Province	Panamic Province
SUBORDER OXYNOACEA (SHELLED SLUGS)				
FAMILY OXYNOIDAE (REDUCED SHELL SLUGS)				
<i>Oxynoe panamensis</i> Pilsbry & Olsson, 1943				X
<i>Lobiger souverbii</i> Fischer, 1856				X
FAMILY JULIIDAE (BIVALVED SLUGS)				
<i>Berthelinia chloris</i> (Dall, 1918)			X	X
<i>Julia thecaphora</i> (Carpenter, 1857) <sup>1</sup>				X
SUBORDER PLAKOBRANCHACEA				
SUPERFAMILY PLAKOBRANCHIOIDEA (PARAPODIA-BEARING SLUGS)				
FAMILY PLAKOBRANCHIDAE				
<i>Elysia hedgpethi</i> Marcus, 1961	X?	X	X	X
<i>Elysia</i> sp. 1 of Behrens (1991)			X?	X?
<i>Elysia diomedea</i> (Bergh, 1894) <sup>2</sup>				X
<i>Elysia oerstedii</i> Mörch, 1859				X
<i>Elysia vreelandae</i> Marcus & Marcus, 1970				X
SUPERFAMILY LIMAPONTIOIDEA (CERATA-BEARING SLUGS)				
FAMILY POLYBRANCHIIDAE				
<i>Polybranchia viridis</i> (Deshayes, 1857) <sup>3</sup>				X
<i>Cyerce ortei</i> Valdés & Camacho-García, 2000				X
FAMILY HERMAEIIDAE				
<i>Aplysioopsis enteromorphae</i> (Cockerell & Eliot, 1905) <sup>4</sup>	X	X	X	X
<i>Aplysioopsis oliviae</i> (MacFarland, 1966)	X?	X	X	
undescribed species <sup>5</sup>			X	
<i>Hermaea vancouverensis</i> O'Donoghue, 1924	X	X	X	
<i>Hermaea hillae</i> Marcus & Marcus, 1967				X
FAMILY LIMAPONTIIDAE				
<i>Alderia modesta</i> (Lovén, 1844)	X	X	X	
<i>Ercolania boodlea</i> (Baba, 1938)			X	X
<i>Ercolania fuscata</i> (Gould, 1870)				X
<i>Olea hansineensis</i> Agersborg, 1923	X	X	X	
<i>Placida dendritica</i> (Alder & Hancock, 1843)	X	X	X	X
<i>Placida</i> sp. 1 of Behrens (1991)			X	
<i>Stiliger fuscovittatus</i> Lance, 1962	X	X	X	X
<i>Stiliger</i> sp. <sup>6</sup>				X
<i>Stiliger</i> sp. <sup>7</sup>				X
Total Number of Species	6–8	8	12–13	18–19

<sup>1</sup> (Synonym: *J. equatorialis* Pilsbry & Olsson, 1944) based on Williams & Gosliner (1973).

<sup>2</sup> Name change by Gosliner (1995).

<sup>3</sup> Synonym: *Phyllobranchillus viridis* (Deshayes, 1857).

<sup>4</sup> Not *enteromorphae* as listed in MacFarland, 1966 (see Marcus & Marcus, 1967; Behrens, 1991a).

<sup>5</sup> Found by M. Chamberlain in southern California (D. Behrens, personal communication, 2000).

<sup>6</sup> Found by Lance & Farmer in the Gulf of California on *Codium magnum* (Farmer, 1996; Lance, 1998; D. Behrens, personal communication, 2000).

<sup>7</sup> Found by Jeff Goddard in the Gulf of California on *C. fragile* (J. Goddard, personal communication, 2000).

? Reflects the uncertainty in dividing lines between biogeographic provinces (see Hartman & Zahary, 1983).

is rather unusual. It may indicate the species' introduction along historical trade routes (Ferreira & Bertsch, 1975) or cryptic species (Burn, 1998; Ellis, 1999; Burn, personal communication to Ellis, 2000). (2) *Ercolania boodlea*

is common on Japanese shores (Baba, 1938; Usuki, 1977; Trowbridge, personal observations) but was only recently reported in the Gulf of California (Farmer, 1980; Behrens, 1991a). This pattern is symptomatic of a recent species

Table 2

Distribution of common sacoglossans on northeastern Pacific shores

Species	Locations	References
<i>Elysia hedgpethi</i>	<b>Alaska:</b> no records <b>British Columbia:</b> Gibraltar Is., Diana Is., Bordelais Islets, Brady's Beach, Grapper Inlet near Bamfield, Vancouver Is.; Brentwood Bay, Vancouver Is. <b>Washington:</b> San Juan Is. <b>Oregon:</b> Boiler Bay & Seal Rock, Lincoln Co.; Coos Bay, Coos Co. <b>California:</b> Tomales Bay Oyster Company mudflats & Richardson Bay, Marin Co.; near Redwood Creek & Port of Redwood City, SF Bay; Pebble Beach, Moss Beach, Park's Point, Pescadero Point, Point Pinos, Monterey Co.; Elkhorn Slough; Shell Beach & Morro Bay, San Luis Obispo Co.; Point Sal, Coal Oil Point, Santa Barbara Yacht Harbor, Carpinteria, Santa Barbara Co.; Flat Rock, Palos Verdes, Los Angeles Co.; La Jolla; Newport Bay, Orange Co. <b>Baja California:</b> Bahía San Quintín, Bahía Tortugas, Bahía de los Angeles, Puertecitos <b>Sonora:</b> Bahía de San Carlos	Lance (1961, 1966), Marcus (1961), Steinberg (1963), MacFarland (1966), Farmer (1967), Sphon & Lance (1968), Greene (1970a, b, c), Roller (1970b), Goddard (1973, 1984), Green & Muscatine (1972), Gosliner & Williams (1973), Williams & Gosliner (1973), Behrens & Tuel (1977), Millen (1980), T. Gosliner in Behrens (1991a), Lance (1998), C. Trowbridge (unpublished data)
<i>Alderia modesta</i>	<b>Alaska:</b> Cordova, Prince William Sound* <b>British Columbia:</b> Neroutsos Inlet near Port Alice, Ladysmith, Pachena Estuary, Louie Bay, Esperanza Inlet, Vancouver Is. <b>Washington:</b> Garrison Bay & False Bay, San Juan Island <b>Oregon:</b> Coos Bay and Charleston, Coos Co.; Yaquina Bay, Lincoln Co. <b>California:</b> Freshwater Slough, Park Street Slough, & Park Street marsh in Arcata Bay, Humboldt Co.; Bodega Bay, Schooner Bay, Drake's Estero, Marin Co.; Bay Farm Is., Alameda Co.; San Francisco Bay; Elkhorn Slough, Monterey Bay; Newport Bay, Orange Co.; San Elijo estuary, Kendall-Frost Marine Reserve and Northern Wildlife Preserve, San Diego River Flood Control Channel, & Mission Bay, San Diego Co. <b>Baja California:</b> San Quintín Bay <b>Other:</b> North Atlantic shores	Hand (1955), Hand & Steinberg (1955), Steinberg (1963), Gosliner & Williams (1973), Williams & Gosliner (1973), Belcik (1975), Thompson (1976), McLean (1978), S. V. Millen (1980, personal communication, 2000), J. Goddard (1984, personal communication, 2000), Jaeckle (1984), Trowbridge (1993c), Lance (1996), Krug (1998b), Krug & Manzi (1999), W. Farmer (personal communication, 1999)
<i>Aplysiopsis enteromorphae</i>	<b>Alaska:</b> Cutter Rock, Ketchikan <b>British Columbia:</b> Crescent Beach; Gambier Is.; Grappler Inlet, Bamfield, & Esperanza Inlet, Vancouver Is. <b>Washington:</b> Argyll Lagoon, Garrison Bay, Wescott Bay, & Mitchell Bay, San Juan Is.; Kayostla Beach <b>Oregon:</b> Boiler Bay & Seal Rock, Lincoln Co.; Neptune Beach & Strawberry Hill, Lance Co.; South Cove, Good Witch Cove, & South Slough, Coos Co. <b>California:</b> Omenoku Pt. & Punta Gorda, Humboldt Co.; Bolinas, Tomales Bay, Bodega Bay; Drake's Estero, Marin Co.; Duxbury Reef, Marin Co.; Scott Creek, Santa Cruz Co.; Elkhorn Slough, Monterey Bay; Cayucos, Hazard Canyon, & Shell Beach, San Luis Obispo Co.; Leo Cabrillo Beach State Park, Los Angeles Co.; Point Sal, Santa Barbara Co.; San Diego; La Jolla Bay, Newport Bay, Orange Co.; Dead Man's Bay, San Pedro <b>Baja California:</b> Bahía de los Angeles, Bahía San Quintín <b>Sonora:</b> Bahía de San Carlos	Cockerell & Eliot (1905), Connor (1961), Lance (1961, 1998), Marcus (1961), Steinberg (1963), MacFarland (1966), Sphon & Lance (1968), Roller & Long (1969), Gosliner & Williams (1970, 1973), Greene (1970a), Williams & Gosliner (1973), Belcik (1975), S. V. Millen (1980, 1989, personal communication, 2000), J. Goddard (1984, 1987, personal communication, 2000), Jaeckle (1984), Behrens (1991a) Trowbridge (1993a, d, personal observations), Goddard et al. (1997), Lance (1998)
<i>Hermaea vancouverensis</i>	<b>Alaska:</b> Humboldt Harbor, Shumagin Islands; Spruce Cape, Kodiak Is.; Cutter Rock & Blank Is., Ketchikan <b>British Columbia:</b> Port Hardy & Newcastle Is., Vancouver Is.; Saturna Is. & Flat Top Is. <b>Washington:</b> Kayostla Beach <b>Oregon:</b> Boiler Bay & Seal Rock, Lincoln Co.; North Cove, Coos Co. <b>California:</b> Bodega Harbor & Coleman State Beach, Sonoma Co.; Duxbury Reef, Marin Co. <b>Baja California:</b> No records	O'Donoghue (1924), Sowell (1949)*, Steinberg (1963), Williams & Gosliner (1973), S. V. Millen (1980, 1983, personal communication, 2000), Goddard (1984), Foster (1987), T. Gosliner in Behrens (1991a), Goddard et al. (1997), Trowbridge (personal observations)



Table 2  
Continued

Species	Locations	References
<i>Placida dendritica</i>	<b>Alaska:</b> Bertha Bay, Chichagof Is. <b>B.C.:</b> Triangle Is., Diana Is., Grappler Inlet, Brady's Beach, Port Renfrew, Bamfield, Vancouver Is.; Mills Bay near Victoria <b>Washington:</b> Kayostla Beach; Cattle Point, San Juan Island <b>Oregon:</b> Boiler Bay, Yaquina Head & Seal Rock, Lincoln Co.; Strawberry Hill, Lane Co.; South Cove, Good Witch Cove, & Squaw Is., Coos Co.; S.H. Boardman State Park, Curry Co. <b>California:</b> Palmer's Pt. & Trinidad Bay, Humboldt Co.; Bodega Head, Sonoma Co.; Duxbury Reef, Marin Co.; Richardson Bay, Marin Co.; Fort Barry Docks, SF Bay; Punta Gorda, Humboldt Co.; Carmel Bay, Park's Pt., Pescadero Point, Point Pinos, & Cypress Point, Monterey Co.; Shell Beach & Morro Bay, Pismo Beach, San Luis Obispo Co.; Flat Rock, Palos Verdes, Los Angeles Co.; Newport Beach and Newport Bay, Orange Co. <b>Baja California:</b> Isla San Benito; Bahía San Quintín, Bahía de los Angeles <b>Sonora:</b> Bahía de San Carlos <b>Other:</b> Japan, north Atlantic, Australasia, South Africa***	Sowell (1949)**; MacFarland (1966), Long (1969), Gosliner & Williams (1970), Greene (1970a), Marcus & Marcus (1970a), Roller (1970b), Greene & Muscatine (1972), Goddard (1973, 1984, 1987, 1990), McLean (1976), Williams & Gosliner (1973), Lambert (1976), Thompson (1976), S. V. Millen (1980, personal communication, 2000), Jaeckle (1984), Gosliner (1987), Behrens (1991a, 1998), Trowbridge (1991a, b, 1995, 1998a, b, 1999, unpublished data), Ichikawa (1993) Goddard et al. (1997), Lance (1998), O'Clair & O'Clair (1998)
<i>Stiliger fuscovittatus</i>	<b>Alaska:</b> Cutter Rock, Ketchikan <b>B.C.:</b> Flat Top Islands (Saturina & Bath); Porlier Pass near Galiano Is.; Stubbs Is. near Telegraph Cove, & Sooke Harbour, Vancouver Is. <b>Washington:</b> San Juan Island <b>Oregon:</b> Seal Rock, Lincoln Co.; Isthmus Slough, Coos Bay, Coos Co. <b>California:</b> Arcata Bay & Humboldt Bay, Humboldt Co.; SF Yacht Harbor, Fort Barry, Sausalito, Marin Co.; Morro Bay Docks & Shell Beach, San Luis Obispo Co.; San Diego & Mission Bays <b>Baja California:</b> Bahía de los Angeles <b>Other:</b> Sebastian Inlet Jetty & Indian River Lagoon at Titusville, Florida	Lance (1962, 1966), Steinberg (1963), Roller & Long (1969), Gosliner & Williams (1970), Case (1972), Williams & Gosliner (1973), S. V. Millen (1980, 1989, personal communication, 2000), Jensen & Clark (1983), Jaeckle (1984), Clark & DeFreese (1987), Trowbridge (1994, unpublished data), Clark (1995), J. Goddard (personal communication, 2000)
<i>Olea hansineensis</i>	<b>Alaska:</b> Cordova, Prince William Sound* <b>British Columbia:</b> Tuwanek Pt., Sechelt Inlet; Sooke Harbour & Tofino, Vancouver Is. <b>Washington:</b> Jackle's Lagoon & Garrison Bay, San Juan Is. & Brown Is. & Parks Bay, Shaw Is. <b>Oregon:</b> No reports <b>California:</b> San Clemente Is.	Agersborg (1923), Gonor (1961), Steinberg (1963), Hurst (1967), Crane (1971), Robilliard (1971), Millen (1980), R. McPeak & D. Mulliner in Behrens (1991a), J. Goddard (personal communication, 2000)

\* New records in preparation: J. Goddard (personal communication, 2000).

\*\* Sowell's (1949) record of *Hermaea vancouverensis* probably refers to *Placida dendritica*; this inference is based on the superficial similarity of the two species, the fact that *P. dendritica* was not recognized on northeastern Pacific shores until the 1960's (MacFarland, 1966; Long, 1969), the author's familiarity with the site in Oregon, and perhaps most importantly because the algal host was specified to be the green alga *Bryopsis corticulans*, not the colonial diatom *Isthmia nervosa*. I do not agree with Belcik's (1975) interpretation that the record was *Aplysiopsis enteromorphae* (as *A. smithi*) because of difference in size, color, tidal level, and algal food.

\*\*\* May be sibling species (C. Trowbridge, work in progress).

introduction, although additional evidence (e.g., confirming the species identity) is needed to support this hypothesis. (3) The appearance of the northeastern Pacific *Stiliger fuscovittatus* in Indian River Lagoon, Florida (Jensen & Clark, 1983; Clark, 1995) seems also to be due to an anthropogenic introduction, particularly as this species feeds on filamentous red algae (*Polysiphonia*) commonly

growing on ship hulls, floating docks, floats, and other "artificial" surfaces.

Chapman & Carlton (1991) suggested a number of criteria to evaluate whether a species was native or introduced. All three of the above cases may represent cases of introductions, although, based on their criteria, additional information is needed. When Marcus (1961) first

Table 3

Distribution of the less common species of northeastern Pacific sacoglossans

Species	Locations	References
<i>Oxynoe panamensis</i>	<b>Baja California:</b> Espíritu Santo Is., Candelero Bay; near La Paz <b>Other:</b> Bocas Is., Province of Bocas del Toro, Panama	Pilsbry & Olsson (1943), Smith (1961), Doty & Aguilar-Santos (1970), Lewin (1970), Keen (1971), Williams & Gosliner (1973)
<i>Lobiger souverbii</i>	<b>Baja California:</b> Espíritu Santo Is., Candelero Bay; Playa Maria & Isla San Jose, Baja del Sur <b>Nayarit, Mexico:</b> Santa Cruz <b>Galapagos:</b> Flamingo Cove, Floreana Island, Galápagos Islands <b>Other:</b> Indian, Pacific, and Atlantic Oceans	Marcus & Marcus (1967, 1970a), Keen (1971), Sphon (1971), Sphon & Mulliner (1972), Baba (1974), Larson & Bertsch (1974), Jensen (1983), Jensen & Clark (1983), Clark & DeFreese (1987), Gosliner (1987), Ichikawa (1993), Gosliner et al., (1996)
<i>Berthelinia chloris</i>	<b>Baja California:</b> Bahía Ballenas & La Paz; Punta Abreojos; La Paz; Puerto Ballandra Bay; Magdalena Bay; Espíritu Santo Is., Candelero Bay, Gulf of California <b>Galapagos:</b> Flamingo Cove, Floreana Island, Galápagos Islands	Keen & Smith (1961), Smith (1961), Kay (1964), Keen (1971), Sphon & Mulliner (1972), Williams & Gosliner (1973), Behrens (1991a)
<i>Julia thecaphora</i>	<b>Baja California:</b> La Paz <b>Mexico:</b> Socorro Is. <b>Other:</b> Panama, Colombia, Ecuador, Peru	Pilsbry & Olsson (1944), Keen (1971), Williams & Gosliner (1973)
<i>Elysia vreelandae</i>	<b>Sonora:</b> San Agustín = El Sahuaral	Marcus & Marcus (1970a, b)
<i>Elysia oerstedii</i>	<b>Costa Rica:</b> Puntarenas	Keen (1971)
<i>Elysia</i> sp. 1	<b>Baja California:</b> Magdalena Bay	T. Gosliner in Behrens (1991a)
<i>Elysia diomedea</i>	<b>Baja California:</b> West of Isla Cerralvo; Islas San Francisco, Espíritu Santo, & Cerralvo; Bahía Las Cruces; Bahía Carisalito; Bahía de Concepcion; Bahía de los Angeles; San Marcus Is., Gulf of Calif.; Puerto Lobos; San José Is. <b>Sonora:</b> Puerto Peñasco <b>El Salvador:</b> Pacific coast <b>Other:</b> To Panama <b>Panama:</b> Venado Isl., off Ft. Kobbe; Deale Beach (Ft. Kobbe Beach), Canal Zone	Bergh (1894), MacFarland (1924), Marcus & Marcus (1967), Dushane & Sphon (1968), Trench et al. (1969), Bertsch (1971, 1973), Keen (1971), Bertsch & Smith (1973), Williams & Gosliner (1973), Ireland & Faulkner (1981), Debelius (1996), Bertsch et al. (1998)
<i>Polybranchia viridis</i>	<b>Baja California:</b> Punta Norte, Isla Cerralvo; Rancho Notri Puerto Escondido, Bahía de Palmas, Punta Colorada, Pulmo Reef, Cabo Pulmo <b>Nayarit:</b> Punta Mita <b>Other:</b> Duncan Is. & Flamingo Cove, Floreana Is., Galapagos; Lesser Antilles; Florida; Panama	Marcus & Marcus (1967, 1970a), Sphon & Mulliner (1972), Bertsch & Smith (1973), Ferreira & Bertsch (1975), Clark (1995), Debelius (1996)
<i>Cyerce orteai</i>	<b>Costa Rica:</b> Playa Cabuya, Cabuya, Cóbano, Puntarenas; Estación San Miguel, Reserva Natural Absoluta de Cabo Blanco, Cabuya, Cóbano, Puntarenas; Playa Ocal del Peñón, Santa Teresa, Cóbano, Puntarenas	Valdés & Camacho-García (2000)
<i>Aplysiopsis oliviae</i>	<b>British Columbia:</b> Saltspring Is. & Brentwood Bay, Vancouver Is. <b>California:</b> Duxbury Reef, Marin Co.; Cabrillo Pt. & Pt. Pinos, Monterey Bay; Santa Barbara Channel, Santa Barbara Co.	MacFarland (1966), Lee & Brophy (1969), Gosliner & Williams (1970), Williams & Gosliner (1973), Millen (1980)
<i>Placida</i> sp. 1	<b>California:</b> San Diego Bay	J. Hamann in Behrens (1991a)
<i>Hermaea hillae</i>	<b>Sonora:</b> Puerto Peñasco	Marcus & Marcus (1967)
<i>Stiliger</i> sp.	<b>Baja California:</b> Bahía San Quintín	Farmer (1996), Lance (1998), D. Behrens (personal communication, 2000)
<i>Ercolania boodleae</i>	<b>California:</b> Mission Bay <b>Sonora:</b> Puerto Peñasco <b>Other:</b> Japan	Baba (1938, 1949), Baba & Hamatani (1952, 1970), Usuki (1977), W. Farmer (1980, personal communication, 1999), J. Hamann in Behrens (1991a)

Table 3  
Continued

Species	Locations	References
<i>Ercolania fuscata</i>	<b>Sonora:</b> near Puerto Peñasco <b>Other:</b> Florida, northwestern Atlantic	Clark (1975, 1995), Ferreira & Bertsch (1975), Jensen & Clark (1983), Clark & DeFreese (1987), Jensen (1988), references therein

described *Elysia hedgpethi*, he considered whether it might have been introduced, given its occurrence on a mudflat in close proximity to mariculture facilities (Pacific oysters) in Tomales Bay, California; after comparing the species to congeners around the world, he concluded that it was a separate species. Soon after its initial description in 1961, *E. hedgpethi* was reported to be widespread (to the north and south); thus, this species exemplifies an overlooked native that differs from geographically distant endemic species (e.g., from the Japanese *E. japonica*, the European *E. viridis*, etc.).

## PREY SPECIES AND FEEDING

### Diets

Sacoglossans are traditionally considered to be stenophagous consumers with comparatively specialized host-plant associations (see Williams & Walker, 1999). For 76% of the northeastern Pacific species, the feeding habits have been generally well characterized, at least to the generic level (Table 4). Difficulty or unresolved taxonomy within some algal groups (e.g., *Vaucheria*, *Cladophora*, *Chaetomorpha*) as well as malacologists' inexperience with distinguishing algal species (e.g., *Codium* and *Polysiphonia*) have hindered progress in this area. For *Hermaea hillae*, *Elysia* sp. 1, *E. oerstedii*, and *Cyerce ortei*, prey species have not been described.

Radulae of many species have been illustrated (O'Donoghue, 1924; Hand & Steinberg, 1955; Gonor, 1961; Lance, 1962; Marcus & Marcus, 1970b; Ferreira & Bertsch, 1975; Gascoigne, 1975; Farmer, 1980; Bleakney, 1989, 1990; Behrens, 1991b; Valdés & Camacho-García, 2000). Jensen (1980, 1993, 1996, 1997) hypothesized that tooth shape is directly related to food type. Jensen's results may assist in determining the diets of the poorly studied species; such extrapolation would be a useful tool for future study of uncommon sacoglossans. Furthermore, Bleakney (1989, 1990) and Jensen (1996, 1997) have reported intraspecific variation in radular tooth morphology in two species on different diets; it would be intriguing to know whether this phenomenon also occurs in *Aplysiopsis enteromorphae*, *Elysia hedgpethi*, and other northeastern Pacific species that feed on two or more genera of algae.

The feeding ecology of northeastern Pacific sacoglossans has been investigated experimentally for only a few species: *Placida dendritica* (Trowbridge, 1991a, b, 1992a, b, 1993b, 1997, 1998a, b), *Elysia hedgpethi* (Trowbridge, unpublished data), *Aplysiopsis enteromorphae* (Gonor, 1961; Trowbridge, 1993a), *Stiliger fuscovittatus* (Case, 1972), *Ercolania fuscata* (on Atlantic shores; Clark, 1975; Jensen, 1983), and *Ercolania boodlea* (on Japanese shores; Usuki, 1977). As Williams & Walker (1999) have emphasized, there is considerable room for improvement in the experimental and statistical rigor of feeding experiments. For example, for feeding preference experiments, some of the needed changes include (1) adequate replication and (2) independence of replicates (individual animals in separate containers making separate and individual choices). For experiments in which groups of animals are maintained on different diets (e.g., Chia & Skeel, 1973), the container or dish is the replicate, not the animals within the dish. For experiments in which groups of animals are placed in a single arena with a pairwise choice of algal species (e.g., Jensen, 1983), the individual slugs are not independent and thus cannot constitute replicates. If experiments involve measuring prey mass loss due to slug herbivory, negative controls should be included in order to measure the endogenous mass loss in the absence of herbivory. Peterson & Renaud (1989) describe the statistical methodology required to test for significant preferences between pairwise choices; multiple-choice experiments are fraught with statistical peril despite their clear biological significance.

Even with the best-studied associations, slug discrimination among, preference of, and performance on congeneric algal species have not been resolved. For example, *Placida dendritica* exhibits distinct feeding preferences among three algal hosts on Oregon shores (*Bryopsis corticulans*, *Codium fragile*, and *C. setchellii*) (Trowbridge, 1991a); on northeastern Pacific shores, there are at least six potential host species, yet there is no information on whether *P. dendritica* consumes *C. ritteri* in Alaska, *C. cuneatum*, *C. hubbsii*, or *C. johnstonei* in southern California, or *C. magnum* and allies in the Gulf of California. Similarly, many authors report that *Stiliger fuscovittatus* consumes *Polysiphonia pacifica*, *Polysiphonia* sp., and *Callithamnion* sp. (Lance, 1962; Stein-

Table 4

Adult diets described for northeastern Pacific sacoglossan opisthobranchs. In many cases, it is unclear if authors are reporting their own observations of diets or merely reiterating previous reports.

Species	Diets	Description of prey	References
<i>Oxynoe panamensis</i>	<i>Caulerpa sertularioides</i> , <i>Halimeda</i>	coenocytic green algae	Doty & Aguilar-Santos (1970), Lewin (1970), Keen (1971)
<i>Lobiger souverbii</i>	( <i>Caulerpa racemosa</i> , <i>C. brachypus</i> , <i>C. paspaloides</i> , <i>C. serrulata</i> )*	coenocytic green algae	Sphon & Mulliner (1972), Baba (1974), Clark & Busacca (1978), Jensen (1983), Clark & DeFreese (1987), Gosliner (1987), Ichikawa (1993)
<i>Berthelinia chloris</i>	<i>Caulerpa racemosa</i> , <i>C. sertularioides</i>	coenocytic green algae	Kay (1964), Sphon & Mulliner (1972), Marcus & Marcus (1970a)
<i>Elysia hedgpethi</i>	<i>Codium fragile</i> , <i>C. setchellii</i> , <i>Bryopsis corticulans</i>	coenocytic green algae	MacFarland (1966), Millen (1980), C. Trowbridge (unpublished data)
<i>Elysia diomedea</i>	<i>Padina</i> and perhaps <i>Spyridia</i>	brown algae	Bertsch & Smith (1973)
<i>Elysia vreelandae</i>	<i>Codium</i>	coenocytic green algae	Marcus & Marcus (1970b)
<i>Polybranchia viridis</i>	<i>Caulerpa racemosa</i>	coenocytic green algae	Bertsch & Smith (1973)
<i>Aplysiopsis enteromorphae</i>	<i>Cladophora columbiana</i> , <i>C. trichotoma</i> , <i>Chaetomorpha linum</i> , <i>C. aerea</i> , <i>Urospora</i> , <i>Rhizoclonium</i>	filamentous green algae	Gonor (1961), Greene (1968, 1970a), Millen (1980), Goddard (1984, 1987), Trowbridge (1993a), Lance (1998)
<i>Aplysiopsis oliviae</i>	<i>Griffithsia pacifica</i> , <i>Polysiphonia hendryi</i>	red algae	Millen (1980)
<i>Hermaea vancouverensis</i>	<i>Isthmia nervosa</i>	diatom	Williams & Gosliner (1973), Foster (1987)
<i>Alderia modesta</i>	<i>Vaucheria</i> spp.	xanthophyte	Hand (1955), Hand & Steinberg (1955), Millen (1980)
<i>Olea hansineensis</i>	<i>Precuthona</i> , <i>Haminoea</i> , <i>Melanochlamys</i> , <i>Gastropteron</i> , <i>Archidoris</i> , <i>Hermisenda</i> , <i>Dendronotus</i>	opisthobranch eggs	Hurst (1967), Crane (1971), Robilliard (1971), Millen (1980)
<i>Placida dendritica</i>	<i>Codium fragile</i> , <i>C. setchellii</i> , <i>Bryopsis corticulans</i> , ( <i>Derbesia</i> , <i>Codium</i> spp., <i>Caulerpa lentillifera</i> , or <i>Halimeda cuneata</i> )*	coenocytic green algae	MacFarland (1966), Long (1969), Greene (1970c), Roller (1970b), Williams & Gosliner (1973), Lambert (1976), McLean (1976), Millen (1980), Gosliner (1987), Bleakney (1989, 1990), Behrens (1991a), Trowbridge (1991a, b, 1992a, b), Ichikawa (1993)
<i>Placida</i> sp. 1	<i>Cladophora</i> **	filamentous green algae	Oakes (1979)
<i>Stiliger fuscovittatus</i>	<i>Polysiphonia pacifica</i> , <i>Polysiphonia brodiaei</i> , <i>P. paniculata</i> , and <i>Callithamnion</i> sp.***	red algae	Lance (1962), Steinberg (1963), Beeman & Williams (1970), Case (1972), Clark & Busacca (1978)
<i>Stiliger</i> sp.	<i>Codium magnum</i>	coenocytic green algae	Farmer (1996), Lance (1998)
<i>Stiliger</i> sp.	<i>Codium fragile</i>	coenocytic green algae	J. Goddard (personal communication, 2000)
<i>Ercolania boodlea</i>	<i>Chaetomorpha</i> , <i>Cladophora</i> ( <i>Ulva</i> , <i>Enteromorpha</i> , <i>Boodlea</i> )*	filamentous green algae	Baba (1938, 1949), Baba & Hamantani (1952, 1970), Usuki (1977), photograph in Behrens (1991a)
<i>Ercolania fuscata</i>	<i>Cladophora</i> , <i>Chaetomorpha</i> , ( <i>Cladophoropsis</i> , <i>Bryopsis</i> )*	filamentous green algae	Clark (1975), Jensen (1983), Clark & DeFreese (1987)

\* Algae in brackets are on Japanese shores (Baba, 1938, 1949, 1974; Ichikawa, 1993), northwestern Atlantic shores (e.g., Clark, 1975; Jensen, 1983; Jensen & Clark, 1983; Clark & Busacca, 1978), or South African shores (Gosliner, 1987).

\*\* Collected on alga; not sure what it feeds on (D. Behrens, personal communication, 2000) but see Oakes (1979).

\*\*\* Not *Microcladia coulteri* mentioned by Beeman & Williams (1970); the confusion undoubtedly arose as the slug species was photographed on *M. coulteri* in the original species description (Lance, 1962) and in Behrens (1991a).

berg, 1963; Beeman & Williams, 1980). Case (1972), however, reported that *S. fuscovittatus* ate three species in San Francisco Bay: *P. pacifica*, *P. brodiaei*, and *P. paniculata*. Given that there about 17 species of *Polysiphonia* reported for California, many of which have recognized varieties, it would be intriguing to know how many algal species actually can be used as host species for *S. fuscovittatus*. Clark (1994, 1995) hypothesized that sacoglossans are particularly vulnerable to environmental or anthropogenic changes, owing to their apparent dependence on specific host plants. Little is known, however, to what extent other algal species can serve as alternate hosts.

A related issue is whether sacoglossans are stenophagous at the local scale but more polyphagous at the regional scale (see discussion by Fox & Morrow, 1981). This could occur if diet specificity is affected by developmental processes (e.g., induction of specific digestive enzymes or tooth morphology by initial diet). Recent work on a European sacoglossan (Trowbridge & Todd, 1998, 2001) indicates that the algal substratum used to induce larval metamorphosis does affect subsequent feeding preferences of post-metamorphic juveniles. The role of genetic variation in feeding preferences has not yet been examined but may contribute to regional variability in diet; extensive work on suctorial insects, the terrestrial analog of sacoglossans, has demonstrated the importance of genetic mechanisms (see, for example, Trowbridge, 1991a; Trowbridge & Todd, 2001).

Particularly surprising is the paucity of information about how native sacoglossans have responded to introduced potential hosts. For example, although the invasive pest alga *C. fragile* ssp. *tomentosoides* has occurred in San Francisco Bay, California since 1973 (Silva, 1979), and the alga is a potential host for *Placida dendritica* and *Elysia hedgpethi*, there have been no published studies on temporal changes in host-plant use with the appearance of a new host plant on Pacific shores. The issue has been investigated on northwestern Atlantic shores (Clark & Franz, 1969; Clark, 1975; Bleakney, 1996), northeastern Atlantic shores (Trowbridge & Todd, 1998, 2001), and Australasian shores (Trowbridge, 1995, 1999). Analogous work is being done on Australian and Mediterranean shores with sacoglossans attacking introduced species of *Caulerpa*. With the appearance of *Caulerpa taxifolia* ("killer algae") in San Diego, California, such issues become more pressing.

Finally, a few cases of slug-algal associations have been reported which may not be related to feeding. For example, *Elysia hedgpethi* is often found either crawling on or depositing its egg masses on the green alga *Ulva* or red foliose algae (e.g., Steinberg, 1963; MacFarland, 1966; Behrens & Tuel, 1977). *Aplysiopsis enteromorphae* in high tidepools selects *Mastocarpus papillatus* (= *Gigartina papillata*) for this purpose (C. Trowbridge, personal observation), and conspecifics on mudflats often oc-

cur on the green alga *Enteromorpha* (which the slugs do not eat, despite the slug's species name; Gonor, 1961). Although the significance of the following has not been generally investigated, Case (1972) reported that eggs of *Stiliger fuscovittatus* develop faster when deposited on algal hosts than on glass or loose in seawater. I have also observed slugs clustering on or under non-food macroalgae, presumably to ameliorate desiccation stress during daytime low tides. Finally, experimental work on a European sacoglossan has demonstrated that larvae will settle and metamorphose on non-host species (Trowbridge & Todd, 2001), and Krug (2001) has documented an analogous situation with larval *Alderia modesta* in California. While we must be careful in inferring trophic associations from the presence of a slug on a particular alga (as emphasized by Jensen), field observations of slugs on algae are important and may reflect either trophic associations or previously overlooked, non-trophic aspects of slug biology.

### Foraging and Feeding Behavior

Comparisons of the sacoglossan literature to that of generalist gastropods and other herbivorous invertebrates (e.g., Hawkins & Hartnoll, 1983) reveal major gaps of study in the sacoglossan field; Williams & Walker (1999) noted gaps as well. At least six key issues have not been explored for northeastern Pacific sacoglossans:

- (1) Frequency of feeding.
- (2) Presence of temporal feeding patterns.
- (3) Extent of long and short-range chemoreception of prey species.
- (4) Importance of algal physiological condition.
- (5) Ecological effects of slug grazing.
- (6) Energetics of slugs.

With respect to the first topic, observational data are meager, and quantitative data are lacking (Williams & Walker, 1999). The northeastern Pacific *Olea hansineensis* reportedly feeds periodically a few times per day (Crane, 1971), whereas *Elysia hedgpethi* feeds continuously (Greene, 1970c). Graves et al. (1979) reported that the digestive lumina of *Alderia modesta* contain chloroplasts, suggesting that the species feeds "regularly," whereas the kleptoplastic *Elysia chlorotica* feeds only periodically. The frequency of feeding is difficult to observe directly, given the small size of sacoglossans and the ventral location of the mouth.

Regarding temporal patterns, two-day feeding experiments with *Placida dendritica*, *Elysia hedgpethi*, and *Aplysiopsis enteromorphae* indicate no clear distinctive tidal or diurnal periodicity (see Trowbridge, 1991a, b, 1993a, b). Weaver & Clark (1981) reported that Atlantic species with functional chloroplasts oriented toward light, whereas aposymbiotic species avoided light. If this pattern were general, then most northeastern Pacific species

Table 5

Type of chloroplast retention of northeastern Pacific sacoglossans. Species with melanic pigmentation generally lack functional kleptoplasty (Clark et al., 1990).

Species	Melanic pigment	Type of kleptoplasty	References
<i>Oxynoe panamensis</i>	—	short-term non-functional retention	Muscantine & Greene (1973), Clark et al. (1990)
<i>Lobiger souverbii</i>	—	short-term non-functional retention	Clark et al. (1990)
<i>Berthelinia chloris</i>	—	short-term non-functional retention	Muscantine & Greene (1973), Clark et al. (1990)
<i>Julia thecaphora</i>	—	short-term non-functional retention	Clark et al. (1990)
<i>Elysia hedgpethi</i>	—	short-term (< 12 h) functional retention	Greene (1970a, b, c), Greene & Muscantine (1972)
<i>Elysia diomedea</i>	—	functional retention	Trench et al. (1969)
<i>Polybranchia viridis</i>	—	short-term non-functional retention	Clark, personal observations in Clark et al. (1990)
<i>Aplysiopsis enteromorphae</i>	+	short-term non-functional retention	Clark et al. (1990); but see Greene (1970a)
<i>Alderia modesta</i>	—	short-term (< 12 hr) functional retention	Graves et al. (1979), Clark et al. (1990)
<i>Placida dendritica</i>	—	intermediate non-functional retention	McLean (1976), Greene & Muscantine (1972), Clark et al. (1990)
<i>Ercolania fuscata</i>	+	no retention	Clark et al. (1990)

(Table 5) should avoid light or perhaps be nocturnal because of the general lack of functional kleptoplasty. Rigorous experiments and field observations are needed to address this temporal-pattern hypothesis. Although numerous studies assert sacoglossan crypsis and susceptibility to predators, there is a noteworthy absence of quantitative data documenting temporal patterns of activity patterns for northeastern Pacific slugs. Similarly, for most intertidal species, it is not known whether slugs feed during emergence and/or submergence. *Elysia diomedea* moves around actively during the day at rates up to 9.5 cm minute<sup>-1</sup> and feeds underwater (Marcus & Marcus, 1967; Bertsch & Smith, 1973). *Alderia modesta* moves around on *Vaucheria* mats in the daytime (Trowbridge, 1994), although night observations have never been made. Slugs burrow into the algal mats with increased emergence time, particularly on warm or bright days (Trowbridge, 1994), and thus appear to be active primarily during submergence.

The role of chemoreception in host-plant location for post-metamorphic slugs and for competent veliger larvae has not been explored for northeastern Pacific species (but see Krug & Zimmer, 2000). Jensen (1982, 1988) investigated the mode of chemoreception for tropical species in Florida, but research on Pacific shores is needed. Limited observations suggest that *Olea hansineensis* moves "randomly" across mudflats, locating opisthobranch eggs by chance or perhaps by extremely short-ranged perception (Crane, 1971). In contrast, the extremely rapid recruitment of *Placida dendritica* to algal transplants on Oregon shores (Trowbridge, 1992a, b, 1998b)

suggests that larvae have extremely acute long-distance host detection. Den Hartog (1959) reported that *Alderia modesta* reacted to chemical stimuli from algal food; in contrast, *Aplysiopsis enteromorphae* and *Ercolania boodlea* do not react when presented with cell sap of their algal food (Gonor, 1961; Usuki, 1977), indicative of little, if any, chemoreception. *Ercolania fuscata* exhibited a positive response to algal homogenates (Jensen, 1988), although the nature of the compounds was not identified nor was the effective distance defined over which the cues operated.

Until we know the nature of the cues inducing larval metamorphosis of sacoglossans and the degree of specificity of such cues (see Krug, 2001; Krug & Manzi, 1999; Krug & Zimmer, 2000a, b), we will not be able to understand fully the extent of the stenophagy of the slugs. For example, it has traditionally been assumed that larval metamorphosis of sacoglossans (and for most opisthobranchs) occurs only in response to prey species of the adults. In laboratory experiments with the Atlantic *Elysia viridis*, however, competent larvae metamorphosed on a variety of macroalgae species, including host and non-host algae (Trowbridge & Todd, 1998, 2001). Furthermore, for northeastern Pacific *Alderia modesta*, some larvae in every lecithotrophic clutch metamorphose immediately with no inductive cue, whereas the remaining larvae delay metamorphosis indefinitely until either encountering *Vaucheria*, or dying (Krug, 2001, personal communication, 2000).

In terrestrial herbivore-plant interactions, the nitrogen status, stress level, and physiological condition of the



plants strongly affect herbivory of stenophagous and polyphagous herbivores (reviewed by Trowbridge, 1998b); in marine associations, however, comparable information is generally lacking for intraspecific variation in herbivory. The two notable exceptions are experimental studies with *Placida dendritica*. First, Trowbridge (1998b) reported that desiccation-stressed algal hosts were attacked more frequently by *P. dendritica* than were unstressed thalli on Oregon (and New Zealand) shores. The basis was not improved food quality or attractiveness to adult slugs; the apparent mechanism was that exudates from stressed algal hosts induced higher rates of settlement and metamorphosis to competent larvae than did exudates from unstressed hosts (Trowbridge, 1998b). Second, Trowbridge (1991a) found that adult slugs exhibited no preferences between mechanically damaged and undamaged algal tissue but did grow faster on algae damaged by grazing conspecifics.

Williams & Walker (1999) reviewed the ecological effect of slug herbivory on algal populations. Presumably, species that do not retain functional chloroplasts cause more grazing damage than species that supplement their nutrition with endosymbiosis or kleptoplasty. To what extent do northeastern Pacific sacoglossans have functional chloroplast retention or kleptoplasty? Most northeastern Pacific species retain chloroplasts for varying lengths of time, but only three species have functional retention (*Alderia modesta*, *Elysia hedgpethi*, and *Elysia diomedea*) and it is short-term for the first two (Table 5). The phenomenon of kleptoplasty has been well reviewed (Clark et al., 1990; Clark, 1992; Williams & Walker, 1999). Yet, details for the majority of Pacific species are lacking or are extrapolated from other geographic regions. Based on the information available, most northeastern Pacific species are strictly heterotrophic, and thus their herbivory may be more important in damaging their host plants than species with multiple modes of nutrition.

Aspects of feeding behavior that have been well studied in one species, *Placida dendritica*, are the patterns and ecological consequences of gregarious feeding (Long, 1969; Clark, 1975; Trowbridge, 1991b). On the algal host *Codium setchellii*, 97.4% of the slugs are group members on Oregon shores; on *C. fragile*, 60.3% of slugs are group members; on *Bryopsis*, slugs do not generally aggregate (Trowbridge, 1991b). Gregarious feeding and intraspecific feeding facilitation documented for *P. dendritica* are unusual in sacoglossans and even in marine herbivores (Trowbridge, 1991b).

Sacoglossan herbivory may substantially reduce algal host populations when slug densities and/or per capita feeding rates are high. For northeastern Pacific species, grazing by *Placida dendritica* and *Stiliger fuscovittatus* may be ecologically important to *Codium* and *Polysiphonia* populations, respectively (Case, 1972; Trowbridge, 1992a, 1993b, 1998b). Case (1972:59) remarked that a "large population of *S. fuscovittatus* apparently can reduce the volume of

*Polysiphonia* to such a degree that food becomes a limiting factor."

The role of epiphytes in determining patterns of sacoglossan attack of host plants has not been well explored. Wahl & Hay (1995) reported that epiphytes could either enhance herbivore attack ("shared doom") or decrease it ("associational resistance"). For *Placida dendritica*, algal hosts of *Codium fragile* with the epiphyte *Ceramium codicola* (specific to *Codium*) were more attractive than hosts free of epiphytes (Trowbridge, 1993b). This may be due to several different processes: (1) epiphytes provide slugs a refuge from predators, (2) epiphytes ameliorate physical conditions (e.g., desiccation stress during emergence and wave force during submergence), (3) slugs attack hosts whose defenses are compromised by epiphytes, and (4) red algal epiphytes may induce larval settlement and metamorphosis (Trowbridge, 1993b). Of the northeastern Pacific sacoglossan species, only two are known to consume epiphytes, namely *Hermaea vancouverensis* that feeds selectively on the epiphytic diatom *Isthmia nervosa* that coats intertidal macrophytes in summer and fall, and *Stiliger fuscovittatus* that eats epiphytic (and non-epiphytic) species of *Polysiphonia*. Given the important ecological roles epiphytes may have in mediating slug-host associations, the ecological function should be explored more fully for different sacoglossans.

In terms of sacoglossan energetics, there has been little comprehensive work for any northeastern Pacific species, although there are data for different aspects for different species. The general equation for the energy budget of an organism is:

$$\begin{aligned} \text{Consumption} &= \text{Production} + \text{Fecundity} \\ &+ \text{Respiration} + \text{Excretion} \\ &+ \text{Secretion.} \end{aligned}$$

There have been several studies that have provided estimates of feeding rates for *Olea*, *Alderia*, *Placida*, and *Aplysiopsis* (Crane, 1971; Trowbridge, 1991a, b, 1992a, 1993a, b). There have been a few calculations from per capita feeding rates to population estimates of slug herbivory (Trowbridge, 1992a, 1993a, b). Activity levels and respiration have not been explored for northeastern Pacific species (but see work by Clark, 1975 on Atlantic *Placida dendritica* and *Ercolania fuscata*); fecundity values (section below) are scarce. Thus, there is insufficient information even for the most abundant sacoglossans to determine energetics. Because sacoglossans are suctorial stenophagous feeders, extrapolations from generalist consumers would provide unrealistic estimates.

## REPRODUCTION, DEVELOPMENT AND GROWTH

### Reproduction

Mating and spawning have been documented for several northeastern Pacific species (Gonor, 1961; Seelemann, 1967;

Baba & Hamatani, 1970; Crane, 1971; Case, 1972; Ferreira & Bertsch, 1975; Millen, 1980; Trowbridge, 1992b, 1993d). The minimum size of mating individuals is surprisingly small. For example, *Olea hansineensis* forms courtship groups when 2 mm long, and reproduction commences at 4 mm (Crane, 1971; Chia & Skeel, 1973). In *Stiliger fuscovittatus*, the minimum size of egg mass production is 3 mm (Case, 1972). Mating generally involves paired copulatory behavior typical of most opisthobranchs. *Alderia modesta*, however, inseminates conspecifics hypodermically (Hand & Steinberg, 1955), as do *Ercolania boodleae* (Baba & Hamatani, 1970) and *E. fuscata* (Gascoigne, 1978). Fertile eggs are produced at least 10 days after copulation for *Stiliger fuscovittatus* (Case, 1972), although the generality for other species is not known. Like other opisthobranchs, sacoglossans store sperm obtained from mating partners; the longevity of these allosperm is not known. Opisthobranch allosperm and ova are mixed during egg mass deposition (reviewed by Hadfield & Switzer-Dunlap, 1984) but specific information for sacoglossans is lacking.

Chia & Skeel (1973) and Seelemann (1967) have reported high fecundity values for *Olea hansineensis* and *Alderia modesta*, respectively. For example, *A. modesta* produces about 1000 eggs per day on European shores. On Californian shores, young adults of *A. modesta* lay approx. one egg mass per day over a 2 to 3 week period in the laboratory; furthermore, there was no difference in the frequency of clutch production for planktotrophic vs. lecithotrophic clutches (P. Krug, personal communication 2000). Case (1972) reported much lower values for *Stiliger fuscovittatus*: about 212–232 eggs per day. Egg masses have been described for several species (Gonor, 1961; Lance, 1962; Hurst, 1967; Greene, 1968; Chia, 1971; Case, 1972).

Deposition of the masses (oviposition) is usually on the host plants or other macroalgae in the habitat (e.g., Gonor, 1961; Lance, 1962; Greene, 1968; Case, 1972). In contrast to juveniles and adults, the egg masses, embryos, and larvae do not contain chloroplasts (Greene, 1968; Trench et al., 1969; Case, 1972; Trowbridge, personal observations); thus, retained chloroplasts are newly acquired by each generation. Over 80% of egg masses laid by *Stiliger fuscovittatus* were produced between 11 pm and 8 am (Case, 1972). The generality of nocturnal deposition is not known.

With the exception of lecithotrophic *Alderia modesta* (Krug, 1998b), all species have small ova with mean diameters between 55 and 95  $\mu\text{m}$  (Table 6). In most cases there is one ovum per capsule; the four exceptions are *Lobiger souverbii*, *Elysia diomedea*, *E. hedgpethi*, and *Stiliger fuscovittatus* (Table 6). Case (1972) reported that none of the embryos with two ova per capsule developed for *S. fuscovittatus*. Three other embryonic details are particularly noteworthy. (1) Embryonic development was 10% faster when egg masses were attached to algal hosts

than when attached to glass or floating freely in seawater (Case, 1972). (2) Embryonic synchrony occurs within individual egg masses of *S. fuscovittatus* (Case, 1972). (3) Hatching rates vary between 95% and 99% for *S. fuscovittatus* (Case, 1972). The generality of these patterns merits further investigation for other northeastern Pacific sacoglossans.

Of the northeastern Pacific species for which data are available, all but one have planktotrophic larvae (Table 6); *Alderia modesta* is poecilogonous and produces both planktotrophic and lecithotrophic larvae (Krug, 1998a, b, 2001; Krug & Zimmer, 2000a). Veligers and shells have been described by Hurst (1967), Greene (1968), Case (1972), Goddard (1984), and Krug (1998a, b). The larval types of the majority of warm-temperate to tropical species have not yet been described. Overall, information on development is available for only half of the known northeastern Pacific species (Table 6).

### Larval Development and Metamorphosis

Strathmann (1987) reviewed larval attributes of northeastern Pacific sacoglossans. None of the species has been raised through its life cycle with the exception of *Alderia modesta* from southern California (Krug, 1998a, 2001; Krug & Manzi, 1999; Krug & Zimmer, 2000a, b) and from Europe (Seelemann, 1967) and *Aplysiopsis enteromorphae* (P. Krug, personal communication, 2000). In fact, only a few sacoglossan species with planktotrophic larvae have been successfully raised through their lengthy larval growth period to larval competency, settlement, and metamorphosis (e.g., Krug & Zimmer, 2000); numerous other attempts have failed (e.g., Case, 1972; Trowbridge, unpublished data). This area of research requires more attention, particularly in terms of the rates of larval growth and the nature and specificity of cues inducing larval settlement and metamorphosis. Larvae of *A. modesta* responded to water-soluble algal cues as well as surface-associated compounds (Krug & Manzi, 1999; Krug & Zimmer, 2000a; Krug, 2001). The little quantitative experimental data on sacoglossans (Trowbridge & Todd, 2001) indicate that the paradigm of metamorphosis only in response to adult prey is a significantly oversimplified view based in large part on insufficient controls to test alternative hypotheses (see Havenhand, 1991; Trowbridge & Todd, 2001).

The spatial and temporal patterns of sacoglossan recruitment have not been extensively examined, particularly at the regional scale. On a local scale (e.g., at a given site), *Placida dendritica* recruited more abundantly to algal hosts transplanted to (1) wave-sheltered coves than on closely adjacent points (Trowbridge, 1992a), (2) desiccation-prone microhabitats than to low-stress ones (Trowbridge, 1998b), and (3) horizontal substratum than to closely adjacent vertical substratum (Trowbridge, unpublished data). Peak recruitment rates are 200–400 slugs

Table 6  
Developmental features of northeastern Pacific sacoglossans

Species	Ovum diameter (μm)	Ova per capsule	Shell length at hatching (μm)	Veliger type	References
<i>Lobiger souverbii</i>	(54.6–66.5)*	Up to 5	no data	type I	Clark & Goetzfried (1978), Clark & Jensen (1981)
<i>Elysia hedgpethi</i>	70	1–2	99, 109 105 ± 20.6	type I	Greene (1968), Strathmann (1987), J. Goddard (personal communication, 2000)
<i>Elysia diomedea</i>	no data	6–14	no data	no data	Bertsch & Smith (1973)
<i>Aplysiopsis enteromorphae</i>	66–70	1	107–113 109 ± 1.7	type I	Gonor (1961), Greene (1968), Goddard (1984), Strathmann (1987)
<i>Aplysiopsis oliviae</i>	no data	1	no data	no data	Millen (1980)
<i>Hermaea vancouverensis</i>	no data	1	no data	type I	Trowbridge (unpublished data)
<i>Alderia modesta</i>	68–80 (70–82)*	1	90–124	type I	Hand & Steinberg (1955), Hurst (1967), Thompson (1976), Clark & Goetzfried (1978), Strathmann (1987), Krug (1998b)
	105	1	186 (190)*	type II	Seeleemann (1967), Krug (1998b)
<i>Ercolania boodleae</i>	?	1	?	?	Baba & Hamatani (1952)
<i>Olea hansineensis</i>	81–120 (capsule)	1	110.7	type I	Agersborg (1923), Hurst (1967), Strathmann (1987)
<i>Placida dendritica</i>	no data (47–77+)* (72.0 ± 5.1)*	1	82–112 (113–127)* 97 ± 15.0	type I	Greene (1968), Kress (1971), Clark (1975), Thompson (1976), Strathmann (1987)
<i>Stiliger fuscovittatus</i>	70–95 (66.5)*	1(–2)	110–150	type I	Lance (1962), Case (1972), Clark & Goetzfried (1978), Strathmann (1987)
<i>Ercolania fuscata</i>	(60, 66.5) (64.5 ± 2.0)*	no data	no data	type I	Clark (1975), Clark & Goetzfried (1978), Clark & Jensen (1981)

( ) \* Indicates from regions other than NE Pacific shores.

algal thallus<sup>-1</sup> month<sup>-1</sup> (Trowbridge, 1992a). Given that most northeastern Pacific species appear to have planktotrophic larvae (Table 6), information about the role of nutrient concentrations, phytoplankton concentrations, and upwelling patterns that affect larval survival, growth, and settlement clearly merits attention (e.g., Trowbridge, 1992b). Many sites appear to have high densities of sacoglossan larvae, not because the benthic algal hosts are more attractive or abundant (Trowbridge, unpublished data) but because of the influence of oceanographic conditions on these factors. For example, Seal Rock and Strawberry Hill, Oregon, consistently have high densities of many species of sacoglossans (as well as other opisthobranchs) every spring and summer (in contrast to what was initially reported by Sphon, 1972); these two sites are recruitment “hot-spots” for many types of larvae including barnacles, mussels, and sacoglossans (Menge, 1992; Menge et al. 1997; Trowbridge, 1992b; B. Menge, personal communication 1996). Such meso-scale ocean-

ographic conditions may account for some of the apparent patchiness of sacoglossan populations.

### Post-Metamorphic Growth

Another enigmatic period in sacoglossan life cycles is the post-metamorphic juvenile stage, particularly the behavior, feeding habitats, and growth rates of juveniles. For *Alderia modesta* on European shores, juveniles (0.8 mm long) feed, rapidly develop cerata, and produce eggs when 10 days old at a length of 3 mm (Seeleemann, 1967). On the shores of southern New England, the life span of *A. modesta* was estimated as 2–6 months (Clark, 1975); information for northeastern Pacific populations is not available. Information on growth of *Olea hansineensis* also indicates a rapid life cycle, with 5 days to reach 1 mm and 2–3 weeks to reach sexual maturity (Crane, 1971; Chia & Skeel, 1973). Clark (1975) and Jensen (1983) recorded the growth of Atlantic populations of *Ercolania fuscata* on multiple algal diets (*Cladophora*,

*Chaetomorpha*, *Cladophoropsis*). Furthermore, Clark (1975) conducted reciprocal feeding experiments to determine the importance of algal source of slugs vs. intrinsic food quality; he also documented that slugs were, on average, larger on *Chaetomorpha* on the shore but more abundant on *Cladophora*. Jensen (1983) also recorded the growth of *Lobiger souverbii* on *Caulerpa racemosa* (on Atlantic shores).

Fretter (1941) described the gut of sacoglossans and suggested that species could ingest large quantities in short periods, given the structure of the gut. If high ingestion corresponds to rapid growth, then sacoglossans have the potential for extremely rapid growth. Based on short-term growth rates of *Placida dendritica*, slugs increase in mass at about 25% body mass per day in the laboratory on *Codium* spp. and at 30–40% on *Bryopsis corticulans* (Trowbridge, 1991a). Growth rates in the field were calculated by outplanting algal hosts (with no slugs) and measuring the body size of recruits after different intervals of time (Trowbridge, 1992a). Based on these data, Trowbridge (1991a) estimated the longevity of *P. dendritica* to be 1–2 months. Case (1972) observed that post-settlement juveniles of *Stiliger fuscovittatus* preferred new growth (i.e., terminal young branches) of *Polysiphonia brodiaei* to older, highly corticated algae, and he also suggested that juveniles could starve in the presence of host plants, presumably if young branches were not available. *S. fuscovittatus* reached sexual maturity in less than 2 months after metamorphosis; slugs reproduced for several months, then died (Case, 1972).

## POPULATION DYNAMICS AND STRUCTURE

### Seasonality

Phenological information for northeastern Pacific sacoglossans is rather meager. For all the northeastern Pacific species, the inferred seasonalities (Figure 2) are based on published collection records, my own collection records (C. Trowbridge, unpublished data), or personal communications (J. Goddard, 2000; S. V. Millen, 2000). More sampling and observations are needed before reliable phenological data are available for the less well-studied 19–20 sacoglossan species. Collection records for species in the Gulf of California and southward may indicate that sacoglossans are present much of the year; quantitative abundance data would assist in the interpretation of presence/absence data.

The most comprehensive and quantitative data are on *Placida dendritica* on Oregon shores (Trowbridge, 1992b) where the species occurred on intertidal algal hosts from April to September with occasional slugs being found in March and October (Figure 2). *Aplysiopsis enteromorphae* also appears to be primarily a spring and summer species on Oregon shores (Goddard, 1984:146, J. Goddard, personal communication, 2000; Trowbridge, 1993a, d). Monthly observations over the course of 1 year

(September 1975 to September 1976) at Scott Creek, Santa Cruz County, California showed that *A. enteromorphae* was present year around in high intertidal, outer coast pools; the species peaked in abundance in September and October and declined sharply in November (J. Goddard, unpublished observations). Egg masses were produced year around but were most abundant in September and October (J. Goddard, unpublished observations). The spring and summer seasonality inferred for *Elysia hedgpethi* (Figure 2) is based on my observations for Seal Rock and Boiler Bay, Lincoln County, Oregon (C. Trowbridge, unpublished data). *Elysia hedgpethi* and its eggs were found on *Codium fragile* in La Jolla, California in January and February 2000 (J. Goddard, unpublished observations). Finally, *Stiliger fuscovittatus* in San Francisco Bay, California was most abundant in fall and early winter (Case, 1972); whether the species exhibits a similar phenology on open-coast shores is not yet known.

Several authors (Miller, 1962; Clark, 1975) have categorized opisthobranchs based on whether they are (1) annual to subannual with multiple generations per year or (2) perennial. Based on seasonality data (Figure 3), this dichotomy is difficult to apply to northeastern Pacific species. *Placida dendritica*, *Alderia modesta*, and *Stiliger fuscovittatus* could be assigned to the first category as they have continual recruitment, rapid growth, and early reproductive maturity (Case, 1972; Trowbridge, 1992a, b, 1993c, d). *Aplysiopsis enteromorphae* is clearly subannual in Oregon with a single generation per year (Trowbridge, 1993a, d). For other species, there is not sufficient information to categorize them. Some authors have suggested that spring to summer seasonal patterns reflect slugs tracking seasonally available algal species. Yet, for *P. dendritica*, *Elysia hedgpethi*, *A. enteromorphae*, *A. modesta*, and *S. fuscovittatus*, the algal hosts are present all year, and thus, the seasonal disappearance is due to constraints other than food limitations. For *Hermaea vancouverensis*, the diatom *Isthmia nervosa* is seasonally abundant with peak densities from July to September on Oregon open-coast shores (Trowbridge, personal observations) and perhaps earlier in California; spatio-temporal variation in diatom abundance throughout the slug's range merits examination. Phenological information for three of the northeastern Pacific species, also found on Atlantic shores, is summarized by Clark (1975) and Bleakney (1996); comparable data for Pacific shores are lacking.

### Sacoglossan Abundance

For most northeastern Pacific sacoglossan species, population density information is qualitative: abundant, common, frequent, rare, etc. (e.g., Lance, 1961; Steinberg, 1963; Sphon & Lance, 1968; Roller & Long, 1969; Roller, 1970b; Williams & Gosliner, 1971; Gosliner & Williams, 1973). Quantitative data are slowly being collected. For pool-dwelling species such as *Aplysiopsis enter-*

Species		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
<i>Placida dendritica</i>	N												
	S												
	G												
<i>Elysia hedgpethi</i>	N												
	S												
	G												
<i>Aplysiopsis enteromorphae</i>	N												
	S												
	G												
<i>Alderia modesta</i>	N				?					?	?		?
	S												
	G												
<i>Stiliger fuscovittatus</i>	N												
	S		?										
	G												
<i>Olea hansineensis</i>	N												
	S												
<i>Hermaea vancouverensis</i>	N												
	S				?								
<i>Aplysiopsis Oliviae</i>	N											?	
	S												
<i>Elysia diomedea</i>	G									?			
<i>Oxynoe panamensis</i>	G												
<i>Berthelinia chloris</i>	G												
<i>Lobiger souverbii</i>	G												
<i>Polybranchia viridis</i>	G												
<i>Cyerce orteai</i>	G												
<i>Ercolania fuscata</i>	G												

Figure 2. Seasonality of northeastern Pacific sacoglossan species in three major regions: N (north) indicates Alaska to Oregon, S (south) indicates California, and G (gulf) indicates the Pacific coast of Baja California, the Gulf of California, and southward to the equator. Shaded cells represent slug presence and ? represents presumed occurrence but no published reports, personal observations, or personal communications. Data from Bergh, 1894; Cockerell & Eliot, 1905; MacFarland, 1924, 1966; Sowell, 1949; Hand & Steinberg, 1955; Gonor, 1961; Keen & Smith, 1961; Lance, 1961, 1962, 1966, 1996; Marcus, 1961; Smith, 1961; Hurst, 1967; Farmer, 1967; Marcus & Marcus, 1967; Dushane & Sphon, 1968; Lewin, 1970; Sphon & Mulliner, 1972; Bertsch, 1971, 1973; Robilliard, 1971; Sphon, 1971; Bertsch & Smith, 1973; Chia & Skeel, 1973; Gosliner & Williams, 1973; Williams & Gosliner, 1973; Larson & Bertsch, 1974; Ferreira & Bertsch, 1975; Lambert, 1976; Behrens & Tuel, 1977; McLean, 1978; Millen, 1980, 1989; Goddard, 1984, 1987, personal communication, 2000; Jaekle, 1984; Foster, 1987; Trowbridge, 1993a, d, 1994, unpublished data; Goddard et al., 1997; Bertsch et al., 1998; Lance, 1998.

*omorphae*, Trowbridge (1993a) reported values of up to 50% of pools at individual sites on Oregon shores. For mat-dwelling species such as *Alderia modesta*, population estimates range from tens to thousands per m<sup>2</sup> (Trowbridge,

1993c, d). Lewin (1970) reported that *Oxynoe panamensis* was abundant at about one slug per m<sup>2</sup>. For sacoglossan species inhabiting separate, upright branching algal hosts, estimates of abundance range up to 70% of hosts for *Pla-*

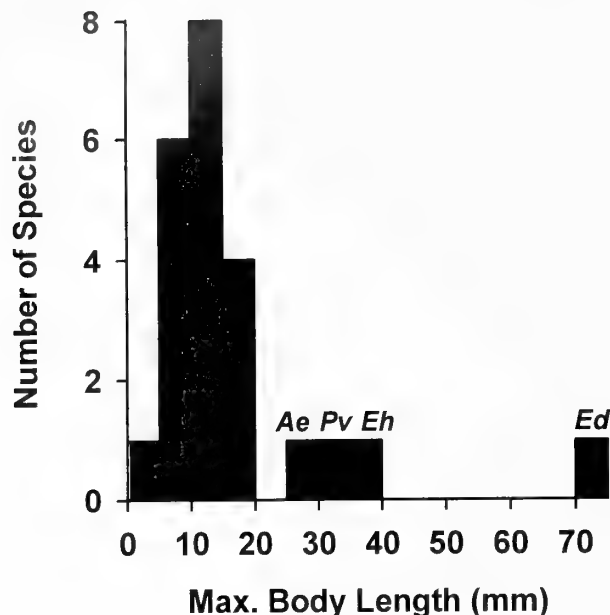


Figure 3. Maximum size–frequency distribution of northeastern Pacific sacoglossans. Abbreviations for the larger species are as follows: Ae = *Aplysiopsis enteromorphae*, Pv = *Polybranchia viridis*, Eh = *Elysia hedgpethi*, Ed = *Elysia diomedea*. Data based on Agersborg, 1923; MacFarland, 1966; Marcus & Marcus, 1967, 1970b; Baba & Hamatani, 1970; Keen, 1971; Chia & Skeel, 1973; Beeman & Williams, 1980; Goddard, 1984; Behrens, 1991a; Trowbridge, 1993a, c; Bertsch et al., 1998; Valdés & Camacho-García, 2000.

*cida dendritica* (Trowbridge, 1993b). Case (1972) reported abundance values of *Stiliger fuscovittatus* in a range of ways: total number of animals found, number of slugs per ml of algal host, and number of slugs per m<sup>2</sup> of substrata. Other authors such as Clark & DeFreese (1987) have reported abundance values as number of slugs per gram dry mass of algae. Dry mass values, however, are not logistically possible for all sites (e.g., marine reserves) or for all algal hosts (e.g., *Vaucheria* spp., which forms mats binding algae and sediments together, the diatom *Isthmia nervosa*, which forms colonies attached to intertidal macrophytes, or *Codium setchellii*, which is relatively scarce (Trowbridge, 1996)). For none of the northeastern Pacific species is there sufficient population data to test whether the positive association between latitude and peak slug density (reported by Clark & DeFreese, 1987 for Atlantic species) also occurs in our region. Given that past collection locations are known for most species (Tables 2, 3), such regional abundance information should be feasible to document.

#### Population Structure

Detailed investigations of population structure have been reported for four species: *Stiliger fuscovittatus* (Case, 1972), *Alderia modesta* (Trowbridge, 1993d),

*Aplysiopsis enteromorphae* (Trowbridge, 1993a, d), and *Placida dendritica* (Trowbridge, 1992b). Detailed length–frequency data (e.g., 871 individuals of *Stiliger fuscovittatus*: Case, 1972) can provide valuable insight into the timing of juvenile recruitment, the rate of juvenile and adult growth, and the timing of adult mortality. When supplemented with environmental data (see Trowbridge, 1992b), population structure data can be a powerful tool for investigating sacoglossan ecology. Because many ecological processes (e.g., fecundity, predation, etc.) are size-dependent, maximum body size of a given species (Figure 3) is valuable and should be included in future collections.

#### ABIOTIC FACTORS

The importance of physical or abiotic factors in structuring sacoglossan populations has been generally assumed but rarely demonstrated. Notable exceptions include descriptive and experimental work by Case (1972) and Trowbridge (1992a, b). The salinity tolerance of *Stiliger fuscovittatus* and *Alderia modesta* presumably affects the spatio-temporal patterns of slug populations within estuaries. Case (1972) reported that 50% of *S. fuscovittatus* individuals tested died within 18 hr when held in 10 ppt water and 50% died within 72 hr at 13 ppt; slugs survived well at salinities of 21 and 33 ppt. Given that salinities in San Francisco Bay dropped to 4 ppt for over 24 hr, Case's suggestion that low salinity caused the dramatic observed decline in slug population density seems well supported. Comparable details have been described for the marsh slug *A. modesta* on European shores (Seelemann, 1968). There was geographic variation in salinity tolerance by *A. modesta*; both high and low salinities disrupted embryonic development and egg production (Seelemann, 1968). Presumably, these autecological responses will dictate species' distributions within bays. Behrens (1980), summarizing the literature for San Francisco Bay, reported that four species occurred in the bay: *A. modesta*, *S. fuscovittatus*, *Elysia hedgpethi*, and *Placida dendritica*. The salinity tolerance of the latter two species is not known, nor is it known for *Aplysiopsis enteromorphae* in high intertidal pools or on estuarine mudflats (Trowbridge, 1993a, d).

The role of fluctuations in air and seawater temperature also merits consideration. Case (1972) reported that adult *Stiliger fuscovittatus* was eurythermal. Both low temperature (4°C) and high (19–23°C) had little effect on adult slugs, despite the narrow temperature range slugs encountered in the bay (11–16°C) (Case, 1972). Furthermore, Trowbridge (1992b) noted that the maximum size of *Placida dendritica* increased significantly with increased seawater temperature. With the availability of temperature chart recorders that could be attached to rocky surfaces on the shores, our understanding of the



contribution of atypically hot or cold days to population fluctuations of slugs should improve.

Finally, the influence of wave exposure on sacoglossan population structure and dynamics has long been surmised. The little information available is mostly indirect. Case (1972) monitored the population abundance of *Stiliger fuscovittatus* along a sea wall with a strong wave exposure gradient; slugs were most abundant at site 2 (site 1 was most sheltered, site 8 was most exposed). He surmised that *S. fuscovittatus* could not persist on the open coast but suggested this hypothesis should be tested experimentally. Other authors, including myself, have subsequently found the species on the open coast, although albeit in comparatively wave-protected habitats (e.g., Seal Rock, Oregon where a series of rocks breaks the wave force considerably). Current techniques now available to measure wave force and water flow in a quantitative fashion could be usefully applied to opisthobranch studies.

## BIOTIC INTERACTIONS

### Predation

Opisthobranchs have a repertoire of defenses against potential predators (e.g., reviews by Thompson, 1976; Di Marzo et al., 1993; Cimino & Ghiselin, 1998; Cimino et al., 1999). For northeastern Pacific sacoglossan species, the support for chemical and/or behavioral defenses is relatively meager (Table 7). Trowbridge (1994) tested the pH of four species of sacoglossans; while they all reduced their surface pH when physically disturbed, only one was acidic (*Aplysiopsis enteromorphae*). Three of the four species were readily consumed by a suite of ecologically relevant predators in spite of any purported defenses such as cerata waving and/or autotomy. However, one of these, *Stiliger fuscovittatus*, was not consumed by the carnivorous *Roboastrea tigris* (Lance, 1997). *Aplysiopsis enteromorphae* was also not consumed by a variety of predators, but the basis of the slugs' unpalatability has not been explored (Trowbridge, 1994).

In a 12-day field experiment conducted in Oregon, *Alderia modesta* abundance was significantly reduced on exposed algal mats and in cage controls compared to in full predator-exclusion cages (Trowbridge, 1994). These results indicate that intense predation did significantly reduce slug populations; moreover, observational evidence from Vader (1981) and experimental evidence from Trowbridge (1993c, 1994) indicates that bird, fish, and crab predation can be important in reducing slug densities. Bleakney (1996) suggested that the "potent, sickly-sweet perfume exuded" by *A. modesta* may be an effective defense against spiders, beetles, and bugs; this intriguing hypothesis merits experimental testing. Predator exclusion experiments need to be conducted for other sacoglossan species, particularly for the warm-temperate to

tropical species that may experience more intense predation than their high-latitude counterparts.

The tropical to subtropical sacoglossan *Elysia diomedea* contains secondary metabolites named tridachione (after the slug's previous genus name *Tridachnella*) and 9, 10-deoxytridachione; it is unclear whether these compounds are derived directly from dietary sources, from retained functional chloroplasts, or synthesized *de novo* (Ireland et al. 1978; Ireland & Faulkner 1981). Vardaro et al. (1992) reported that *Placida dendritica* on Mediterranean shores produced polypropionate compounds *de novo* and that they were not localized in specific tissues. Furthermore, Di Marzo et al. (1993) reported that the Mediterranean *P. dendritica* was unpalatable to fishes; in contrast, Trowbridge (1994) reported that fishes readily consumed specimens on Oregon shores. The source of this variation is intriguing; whether it is due to cryptic species (morphologically similar species that are often confused as a single species), geographic variation in capacity to synthesize compounds *de novo*, or to other factors is not known. The source of the secondary metabolites, however, is seemingly not the algal diet (Cimino et al., 1999).

Information on the predation of sacoglossan larvae and egg masses is meager. Caprellid amphipods may prey upon swimming veliger larvae or settling juveniles (Kae-stner, 1967, cited by Case, 1972). There are some details of egg predation by the sacoglossan *Olea hansineensis*. Crane (1971:58) wrote that "adults were observed to recoil violently from their own egg mass. . . . Apparently, contact is necessary for recognition of their egg masses." This aversion response is not only to their egg masses but also to those of conspecifics (S. V. Millen, personal communication, 2000).

In all of the cases above, the slugs are consumed directly by predators. Predation, however, can have indirect effects on slugs (Case, 1972) when consumers (e.g., birds) consume the substrata (e.g., mussels) upon which the slugs' algal food grows. Indirect effects of predators or generalist herbivores would be most important in cases in which the slugs' algal hosts are epibionts, growing on top of a shellfish or an alga. The importance of this mechanism in regulating slug populations has not yet been explored.

### Competition

In terrestrial systems, there is extensive theoretical and empirical information on the role of interspecific and intraspecific competition among stenophagous herbivores such as phytophagous insects. In marine systems, the topic has barely been addressed. In many cases, sacoglossans occur on host plants not used by other grazers (Trowbridge, 1992a). In other cases, such as *Aplysiopsis enteromorphae*, the slugs coexist with prosobranch gastropods and small crustaceans (e.g., amphipods, isopods) (Trow-

Table 7  
Potential defensive adaptations of northeastern Pacific sacoglossans and the animals' palatability to predators

Sacoglossan species	Potential defenses	Palatability	References
<i>Oxynoe panamensis</i>	algal metabolite sequestration (caulterpin & caulpericin), toxic secretion, tail autotomy	toxic secretion lethal to fish	Doty & Aguilar-Santos (1970), Lewin (1970)
<i>Lobiger souverbii</i>	discharge white substance; lobe autotomy	toxic secretion lethal to fish	Kay (1964), Gosliner (1987), Gosliner et al. (1996)
<i>Elysia hedgpethi</i>	no data	not consumed by fishes, crabs, or <i>Roboastra tigris</i>	Trowbridge (1994), Lance (1997)
<i>Elysia diomedea</i>	tridachione & 9, 10-deoxytridachione (secondary metabolite)	no data?	Ireland et al. (1978), Ireland & Faulkner (1981)
<i>Aphysiopsis enteromorphae</i>	wave & shed cerata; reduce body pH from neutral to pH = 4	unpalatable to fishes and crabs; source not known	Trowbridge (1994)
<i>Alderia modesta</i>	reduce body pH but still basic; peculiar, sickly sweet smell	palatable to fishes & crabs	Hand & Steinberg (1955), Trowbridge (1994), Bleakney (1996)
<i>Ercolania boodleae</i>	exude milky-white fluid	no data?	Baba & Hamatani (1970)
<i>Placida dendritica</i>	wave & shed cerata; reduce body pH when disturbed from basic to neutral; Mediterranean animals synthesize polypropionates <i>de novo</i> (e.g., placidene-A)	Oregon animals palatable to fishes & crabs; Mediterranean animals unpalatable to fishes	Vardaro et al. (1992), Di Marzo et al. (1993), Trowbridge (1994), Cimino et al. (1999)
<i>Stiliger fuscovittatus</i>	wave & shed cerata; reduce body pH when disturbed from basic to neutral	palatable to fishes & crabs; not consumed by <i>Roboastra tigris</i>	Trowbridge (1994), Lance (1997)

bridge, 1993a). On northeastern Pacific shores, *Placida dendritica* and *Elysia hedgpethi* coexist on the same host species and even occasionally on the same thalli (D. Behrens, personal communication, 2000; C. Trowbridge, personal observations); whether the two species facilitate one another, inhibit one another, or have no effect deserves future consideration.

Finally, for the two species that often occur at very high densities, *P. dendritica* and *Alderia modesta*, intra-specific interactions merit investigation. Trowbridge (1991b) demonstrated that small individuals of *P. dendritica* inhibit the feeding and growth of large conspecifics (i.e., intraspecific competition); in contrast, large slugs facilitate the feeding and growth of small and large conspecifics (facilitation). For *A. modesta*, population density and body size are inversely related on a regional scale (Trowbridge, 1993c, d). Whether this pattern reflected intraspecific competition or other mechanisms (e.g., high recruitment coupled with high mortality) has not been investigated.

### SPECIES IDENTITY AND VARIATION

The majority of sacoglossans are described based on morphology, especially radular tooth shape and size and various attributes of the reproductive system. In few cases has the biological species concept been tested by controlled matings of seemingly conspecific animals. Thus, it is frequently difficult to determine whether observed variation is best considered inter-specific or intra-specific. Molecular techniques offer independent methods to test the validity of morphospecies. To date, extremely few molecular studies of this sort have been conducted on sacoglossans (or other opisthobranchs). Theisen & Jensen (1991) investigated genetic variation of European species (including the species *Alderia modesta*) with allozymes. Krug (1998a, b) and Trowbridge (unpublished data) investigated variation in the mtDNA gene cytochrome oxidase subunit I (COI) of *A. modesta*, *Placida dendritica*, and *Ercolania boodleae*. Tholleson (1999) and Trowbridge (work in progress) have sequenced portions of the mtDNA gene 16S rRNA in *P. dendritica*. I predict that molecular techniques will result in the splitting of cosmopolitan species (e.g., *Placida dendritica*) into sibling species, analogous to work on other taxa (e.g., Knowlton, 1993; Geller et al., 1997). *P. dendritica* has been a controversial species for decades (Thompson, 1973, 1976, 1988; Gosliner, 1987; Bleakney, 1989, 1990; Trowbridge, 1997; Burn, 1998; references therein) due to morphological, physiological, and ecological variation. Molecular details may soon resolve whether *P. dendritica* is the appropriate name for the northeastern Pacific species: the species was described from specimens collected from Torbay, England.

Molecular techniques may resolve many of the problematic taxonomic issues as well as assist with the as-

signment of new undescribed species to correct genera. The placement of many species in the families Hermaeiidae and Limapontiidae (*sensu* Jensen, 1996) has been very problematic, rendering the sacoglossan literature difficult to follow. For example, the species *Aplysiopsis enteromorphae* has been placed in three different genera since 1905; many other species have been transferred between genera (in different families) as our understanding has improved. Molecular research may assist in resolving future taxonomic difficulties (e.g., with the undescribed species shown in Table 1). Finally, molecular information could contribute substantially to the questions of larval dispersal, gene flow host specificity, and other ecological or evolutionary issues.

### PROSPECTUS

The field of sacoglossan biology is wide open with extensive areas of unexplored issues. Some of the priority areas for future research on northeastern Pacific sacoglossans are:

1. Investigate the northeastern Pacific sacoglossan fauna from the Gulf of California south to the equator. The investigation of this tropical to subtropical region will undoubtedly raise our estimate of sacoglossan species richness.
2. Document the population structure and dynamics of species, including the seasonality of slug and spawn occurrence, adult immigration, and frequency of adults over-wintering. If sacoglossans are indeed particularly susceptible to environmental degradation, habitat loss, and anthropogenic change (Clark, 1994), basic population information about these species is essential to provide a baseline from which to evaluate future change.
3. Document the host-plant associations in more comprehensive detail, both spatially and temporally. Even specialized associations change on ecological and evolutionary time scales. Thus, understanding the conditions under which trophic flexibility does occur is biologically significant.
4. Study the feeding and foraging behavior, particularly the existence of any temporal patterns (tidal or diurnal/nocturnal). Ecological theories about feeding specificity are frequently based on assumptions of vulnerability of slugs to potential predators; basic information about when and where sacoglossans feed is crucial to evaluating their risk to visual predators.
5. Investigate the relative importance of kleptoplastic vs. heterotrophic sources of nutrition. The new technique of PAM fluorometry provides us with the means of rapidly and non-invasively screening slugs for photosynthetic activity (S. Williams, 2000; personal communication, 2000).
6. Quantify patterns of larval growth, metamorphosis, post-larval growth, and fecundity.

7. Document the effect of physical variables such as salinity, temperature, irradiance, turbidity, etc., as well as UV light, pesticides, and other types of coastal pollution on slug population dynamics (see Clark, 1975, 1994, 1995).
8. Investigate the patterns of genetic variation within and among species; such molecular techniques will aid the correct placement of the taxonomically challenging, undescribed sacoglossans on northeastern Pacific shores as well as contribute to the understanding of sacoglossan population structure and dispersal.

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## *Trogloconcha*, A New Genus of Larocheine Scissurellidae (Gastropoda: Vetigastropoda) from Tropical Indo-Pacific Submarine Caves

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**Abstract.** A new genus *Trogloconcha* is proposed for *Trogloconcha ohashii*, sp. nov. within the Larocheinae Marshall, 1993, a unique subfamily that lacks an anal slit or foramen among the Scissurellidae (Vetigastropoda). The new species inhabits gloomy to totally dark, shallow-water submarine caves and deep caverns and is distributed widely in the tropical to subtropical eastern Indian and western Pacific oceans. *T. ohashii*, sp. nov. has papillate cephalic tentacles unlike species of *Larochea* (the condition is unknown for *Larocheopsis*), has a small operculum, and does not show sexual dimorphism in shell features unlike species of *Larochea* and *Larocheopsis*. One modern species *Trogloconcha tessellata*, sp. nov. from Okinawa, Japan, and one late Oligocene species *Larocheopsis marshalli* Lozouet, 1998, from France, both represented by shell alone, are assigned to the new genus.

### INTRODUCTION

Mollusks from submarine caves primarily accessible through SCUBA diving have been investigated in recent years. Submarine cave mollusks have been most extensively sampled in the Indo-Pacific Oceans (Kase & Hayami, 1992; Hayami & Kase, 1992, 1993, 1996; Kase, 1998a, b, c, 1999; Kase & Kano, 1999; Kano & Kase, 2000) and in the Mediterranean Sea (Di Geronimo et al., 1993, 1997; La Perna, 1998). The investigation of cave mollusks has produced interesting questions, both taxonomically and biogeographically. New mollusks of little known and unknown taxa, and many living features of interesting species previously known only as empty shells have been discovered (Kase & Hayami, 1992; Kase, 1998a, b, c, 1999; Kase & Kano, 1999; Kano & Kase, 2000).

In this paper, a new genus and two new species of the scissurellid subfamily Larocheinae are described. A total of over 1500 specimens of the new species *Trogloconcha ohashii*, mostly represented by empty shells, were collected from a number of submarine caves, caverns, and crevices in the tropical western Pacific and eastern Indian oceans. The habitats are from 4 m to 51 m in depth, gloomy to totally dark inside, mostly filled with calcare-

ous muddy sand, and may have been formed mostly during the low sea levels during the late Quaternary. *Trogloconcha tessellata*, on the other hand, is known only from empty shells at a single locality.

### MATERIALS AND METHODS

Shell-bearing bottom sediments were collected by hand and/or hand-made sampler and sieved with 0.5 mm mesh. The shells were picked under a binocular microscope. In spite of an abundant occurrence of empty shells, live specimens are rare and are represented only by 13 specimens of *T. ohashii*. They have been found only in submarine caves, and have not been found in nearby shallow-water bottoms outside of the caves, nor have they been recorded from deep waters. It seems, however, that the species is not restricted to cave habitats, but is living in coral rubble deeply embedded in inaccessible sublittoral situations. Live animals were collected by brushing the under-surface of coral rubble on the bottom sediments. In "Cross Hole" of Irabu islet of the Miyako Islands, live specimens were collected, together with a number of live bivalves and gastropods that are associated with patchy colonies of a tube-forming annelid. The bivalves are *Cosa waikikia* (Dall, Bartsch & Rehder, 1938), *Chlamydella incubata* Hayami & Kase, 1993, *Chlamydella tenuissima* Hayami & Kase, 1993, etc., which attach to the annelid tubes with byssus. The associated gastropods are scissurellids such as *Scissurella staminea* A. Adams, 1862, and

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undetermined species of *Scissurella*, *Sinezona*, and risoids.

Live animals were relaxed in 7.5% magnesium chloride, fixed in 10% formalin for 24 hours, and preserved in 75% ethanol. For SEM observation of soft parts, animals were gradually dehydrated, transferred to t-butyl alcohol, and dried with a freeze-drier JEOL JFD-300. Radulae were removed from the buccal mass, soaked in sodium hypochlorite for several minutes, then washed, mounted, and dried.

## SYSTEMATICS

Superorder VETIGASTROPODA Salvini-Plawen, 1980

Family SCISSURELLIDAE Gray, 1847

Subfamily LAROCHEINAE Finlay, 1927

Genus *Troглоconcha* Kase & Kano, gen. nov.

**Type species:** *Troглоconcha ohashii* Kase & Kano, sp. nov.

**Diagnosis:** Shell minute, thin, fragile, umbilicate, laterally expanded turbiniform, without anal slit or foramen; aperture large, inner lip simple, without internal inner septum; protoconch almost smooth; teleoconch whorls rounded, with reticulate sculpture. Radula rhipidoglossate with formula  $\infty + 5 + 1 + 5 + \infty$ ; central tooth broadest, lateral 1 broad, laterals 2–4 with slender shafts bowed outwardly near base, lateral 5 with quadrangular broad base, tapered to tip. Operculum rudimentary, with diameter of  $\frac{1}{4}$  of shell aperture. Animal with papillate cephalic and non-papillate epipodial tentacles, without brood pouch. Gonochoristic, no size differences between sexes.

**Etymology:** The genus name is from the combination of Latin, *Troglo* (cave) and *concha* (shell), referring to the habitat of the type species.

**Discussion:** Finlay (1927) established the family Larocheidae to accommodate the single species *Larocha miranda* Finlay, 1927, from off northern New Zealand, and tentatively placed the family close to the caenogastropod family Merriidae (= Vanikoridae). Marshall (1993) examined the shells and radulae of *Larocha* and his new genus *Larochopsis*, and correctly placed the taxon as a subfamily of the Scissurellidae. He defined the subfamily as having these characters: (1) a minute, thin, haliotiform-turbiniform teleoconch with fine reticulate (*Larocha*) or almost smooth (*Larochopsis*) sculpture, without anal slit or foramen; (2) a smooth (*Larochopsis*) or finely granulate (*Larocha*) protoconch; (3) a radula with the formula  $\infty + 5 + 1 + 5 + \infty$ ; (4) animal with right ctenidium absent, left ctenidium monopectinate (*Larocha*); and (5) cephalic and epipodial tentacles non-papillate (*Larocha*).

The new genus *Troглоconcha* is erected for *T. ohashii*, sp. nov. as the type species. One modern species *T. tes-*

Table 1

Gonad analysis of *Troглоconcha ohashii* sp. nov. Shell size is measured maximum shell diameter when shell aperture is placed on flat surface.

Shell size (mm)	Gonad	Locality
1.30	testis	Miyako island, Japan
1.20	ovary	Miyako island, Japan
1.00+	ovary	Miyako island, Japan
1.00	ovary	Miyako island, Japan
0.75	ovary	Saipan

*selata*, sp. nov. from Okinawa, Japan, and one Oligocene species *Troглоconcha marshalli* (new combination, = *Larochopsis marshalli* Lozouet, 1998) from France, both represented by shell alone, are allocated to the new genus on the basis of the overall shell similarity to *T. ohashii*. The shell, radular, and ctenidial characters indicate that the new genus is a member of the Larocheinae (Figures 1–3). However, there are some differences that warrant separating *T. ohashii* from the other larocheinine species at the generic level. The new genus has a smooth protoconch like *Larochopsis* (Figure 2), but differs from the latter in having reticulate teleoconch ornamentation (Figure 1). In radular morphology, *T. ohashii* has a fifth lateral tooth which is more slender than that of *Larocha*, and has a first lateral tooth more slender than that of *Larochopsis* (Figure 3D). Moreover, *T. ohashii* has a rudimentary operculum and micropapillae on cephalic tentacles (Figure 3A–C), whereas the operculum is absent in *Larocha* and *Larochopsis*, and the tentacles are not papillate in *Larocha* (the condition is unknown for *Larochopsis*) (B. Marshall, 1993, personal communication).

Reproductive traits further distinguish the new genus. In *Larocha*, young are brooded in the right subpallial cavity, and the shells exhibit sexual dimorphism: the female has a large internal inner lip septum, whereas there is a small internal inner lip septum in the male. In *Larochopsis*, an internal inner lip septum is absent, and the male seems to be smaller than the female and firmly attaches to the body whorl outside of the parietal area (Marshall, 1993). *T. ohashii*, on the other hand, has neither the internal inner lip septum nor the brooded young in the subpallial cavity, and it lacks the dwarf males on the body whorl of the large female shells. A serial thin section analysis of the gonads reveals either testis or ovary in fully grown specimens of *T. ohashii*. Thus, the species is, like *Larocha secunda* Powell, 1937, and *Larocha scitula* Marshall, 1993, evidently gonochoristic and has males and females of similar shell size (Table 1).

*Troглоconcha ohashii* Kase & Kano, sp. nov.

(Figures 1A–C, 2A, B, 3)

*Larocha miranda* Finlay, Bandel, 1998:66, pl. 33, figs. 3, 4; non Finlay, 1927.

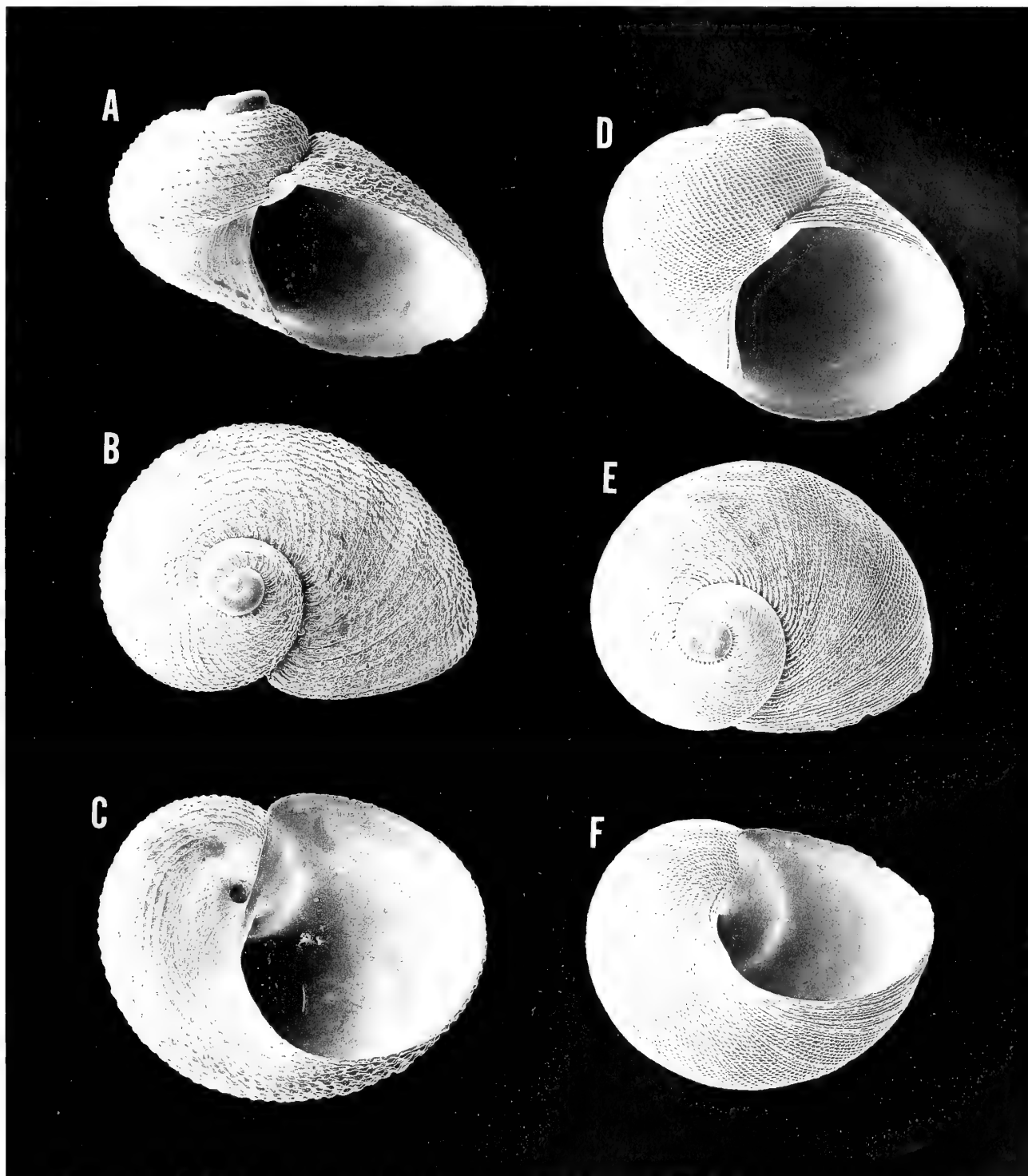


Figure 1. A–C. *Trogloconcha ohashii* Kase & Kano, gen. et sp. nov. Frontal, apical, and basal views of holotype (NSMT Mo72828), 1.13 mm wide, 0.81 mm high, from “Cross Hole,” Irabu islet, Miyako Islands, Okinawa, Japan. D–F. *Trogloconcha tessellata* Kase & Kano, gen. et sp. nov. Frontal, apical, and basal views of holotype (NSMT Mo72830), 1.1 mm wide, 1.02 mm high, from north of Kohama Island, Yaeyama Group, Okinawa.

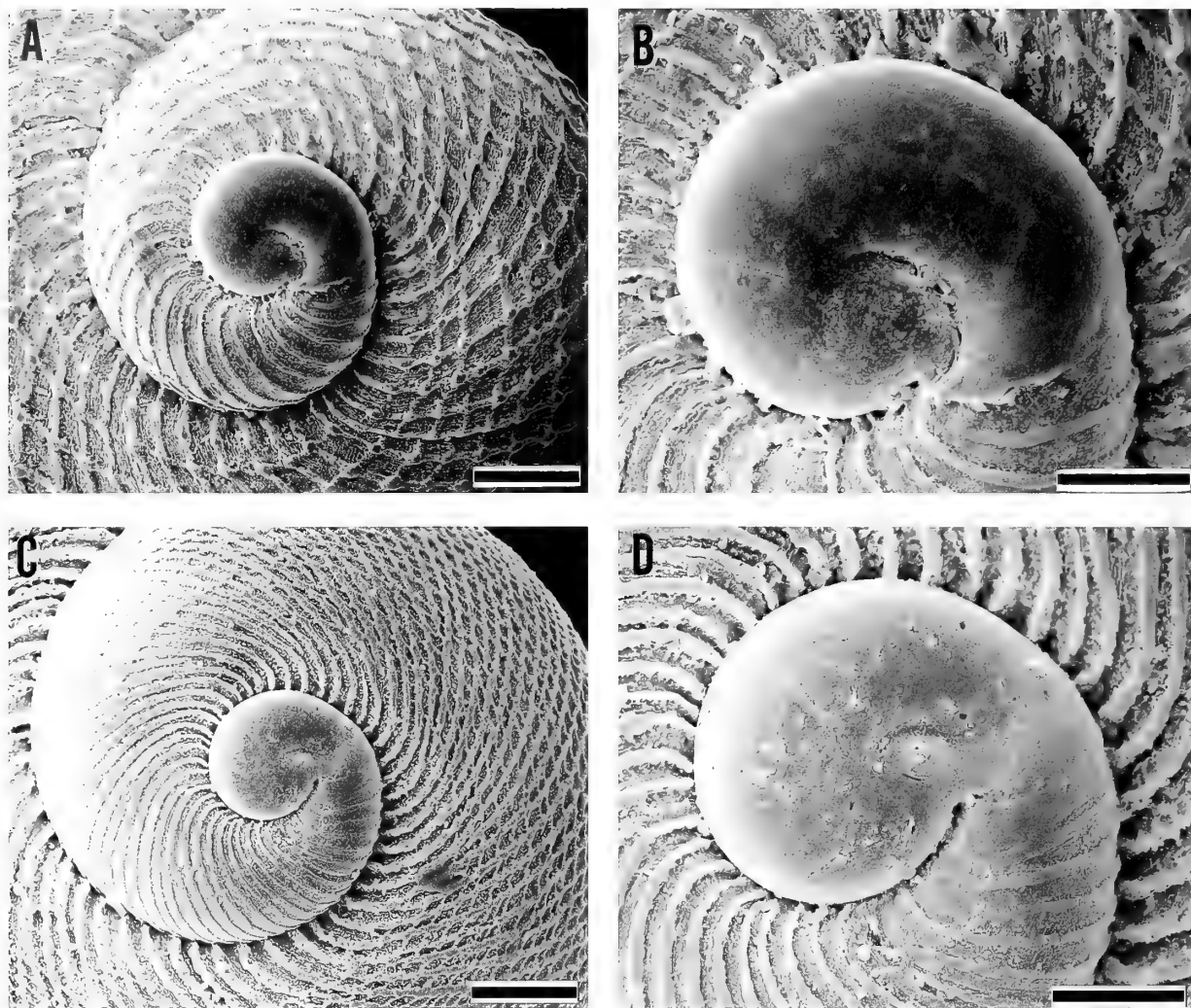


Figure 2. Protoconchs of *Troглоconcha* in apical views. A, B. *Troглоconcha ohashii* Kase & Kano, gen. et sp. nov., holotype, NSMT Mo72828. C, D. *Troглоconcha tessellata* Kase & Kano, gen. et sp. nov., holotype, MSMT Mo72830. Scale bars = 100  $\mu$ m for A and C, and 50  $\mu$ m for B and D.

**Type specimens:** Holotype NSMT Mo72828, 0.81 mm high, 1.13 mm wide; paratypes NSMT Mo72829; paratypes MNHM.

**Type locality:** “Black Hole” diving site, northwest of Shimoji Island, Miyako Group, Okinawa (24°49.1'N, 125°08.3'E); depth 35 m, totally dark inside; calcareous muddy sand.

**Distribution:** This species is widely distributed in the shallow waters of the tropical and subtropical areas between Cocos Keeling (Indian Ocean) in the west and French Polynesia (western Pacific Ocean) in the east.

**Other material examined:** COCOS KEELING—1 empty shell from sta. CK1, cavern, west of West Island, 12°10.8'S, 96°48.8'E, depth 48.6 m, gloomy. CHRISTMAS ISLAND (INDIAN OCEAN)—3 empty shells from

sta. XM4, “Thunder Dome” diving site, long cave seemingly connected to land cave(s), north of Christmas Island, depth 9 m, totally dark. BALI, INDONESIA—20 empty shells, Menjangan Island National Park, south side at “Underwater Cave” dive site, shallow crevice, depth 25–30 m. BORNEO, MALAYSIA—116 empty shells from sta. CT, “Turtle Cavern” diving site, long cave, Sipadan island, Sulu Sea, 118°36.5'E, 05°04.8'N, depth 15 m, totally dark. PHILIPPINES—100+ empty shells from sta. AN4, cavern in front of Vistamar Resort, Anilao, Batangas, 13°45.1'N, 120°55.0'E, depth 40 m, gloomy; 38 empty shells from sta. AN3, “Mapatin Cave” diving site, cave, southwest of Maricaban Island, Batangas, 13°40.2'N, 120°49.0'E, depth 46 m, totally dark; 39 empty shells from “Marigondon Cave” diving site, huge cave, south of Mactan Island, 10°15.8'N, 123°59.2'E,

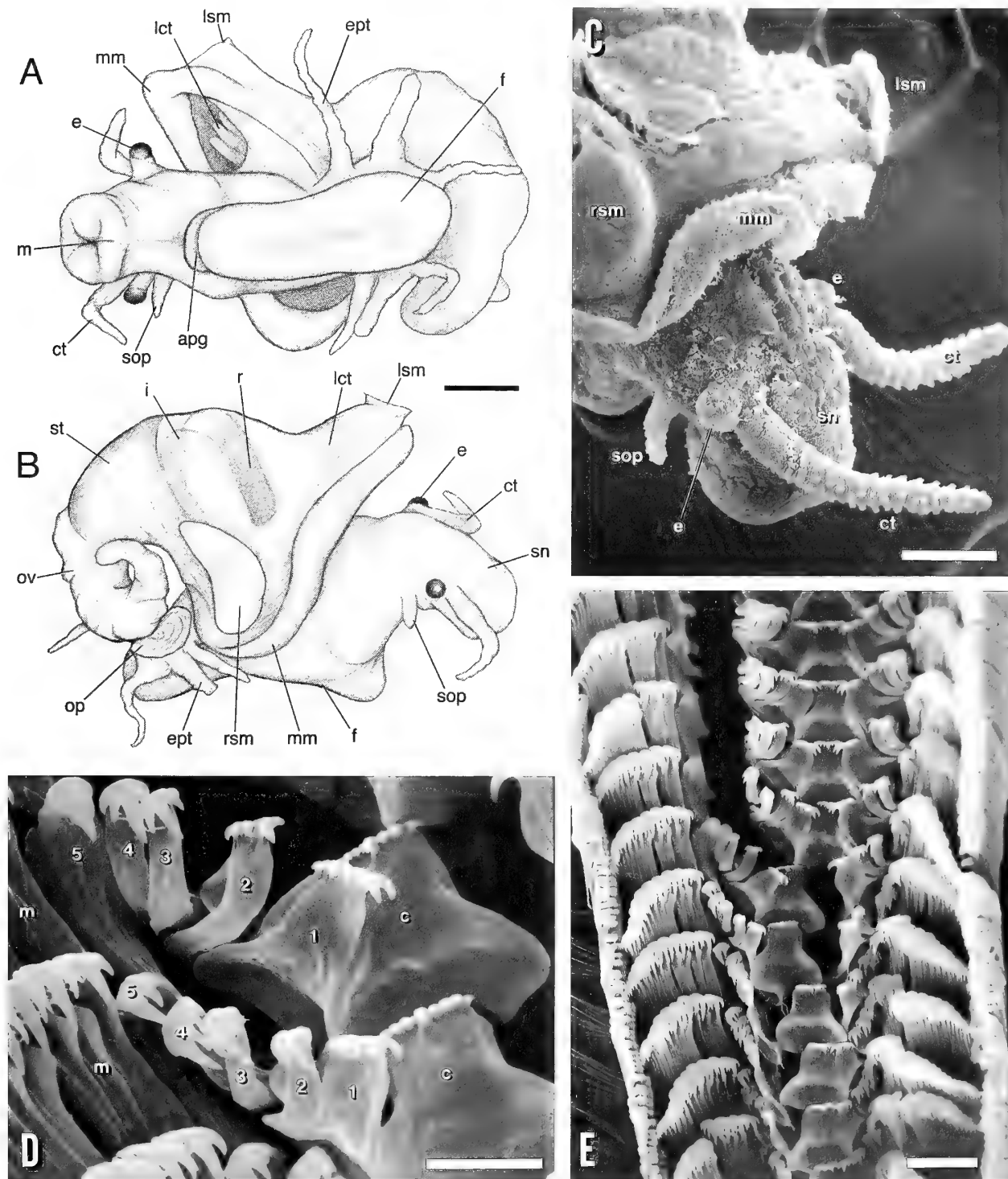


Figure 3. External anatomy and radula of *Troглоconcha ohashii* Kase & Kano, gen. et sp. nov. A, B. Drawings of a female animal from Saipan, removed from shell. Scale bar = 100  $\mu$ m. A. Ventral view. B. Dorsal view. Abbreviations: apg, opening of anterior pedal gland; ct, cephalic tentacle; e, closed eye; ept, epipodial tentacle; f, foot; i, intestine; lct, single left ctenidium; lsm, left shell muscle; m, mouth; mm, mantle margin; op, operculum; ov, ovary; r, rectum; rsm, right shell muscle; sn, snout; sop, right subocular peduncle; st, stomach. C. SEM shot of extracted animal from Miyako Islands, Okinawa. Note papillate cephalic tentacles and a subocular peduncle. Scale bar = 100  $\mu$ m. D, E. Radula of a specimen from Miyako Islands, Okinawa. D. Enlargement of central (c), lateral (1–5), inner marginal (m) teeth, scale bar = 5  $\mu$ m. E. Whole teeth of transverse rows, scale bar = 10  $\mu$ m.



depth 27 m, totally dark; 86 empty shells from two caverns, Balicasag Island of Bohol, 09°32.7'N, 123°40.7'E, depth 17 m, gloomy. OKINAWA ISLANDS, OKINAWA, JAPAN—112 empty shells from "Shodokutsu" (= small cave) diving site, cave, east of Ie islet, 26°42.9'N, 127°50.1'E, depth 20 m, totally dark; 4 empty shells from "Daidokutsu" (=large cave) diving site, huge cave, east of Ie islet, 26°42.9'N, 127°50.1'E, depth 20 m, totally dark. MIYAKO ISLANDS, OKINAWA, JAPAN—43 empty and 3 live shells from "Cross Hole" diving site, cave, Irabu islet, 24°51.6'N, 125°09.5'E, depth 15 m, gloomy; 3 empty shells from "Lunch Hole," cave in tidal flat, Irabu Islet, 24°51.6'N, 125°10.0'E, depth 4 m, totally dark; 1 empty shell from "L-arch" diving site, L-shaped cave, Irabu islet, 24°51.7'N, 125°09.7'E, depth 25 m, totally dark; 6 empty shells from "Devil's Palace" diving site, long cave, Shimoji islet, depth approx. 25 m, gloomy; 107 empty shells from "Witch's House" diving site, cave, Shimoji islet, 24°49.3'N, 125°08.3'E, depth 35 m, totally dark; 3 empty shells from "Toriike" diving site, approx. 30 m long tunnel, Irabu islet, 24°49.1'N, 125°08.3'E, depth 12 to 40 m, gloomy. YAEYAMA ISLANDS, OKINAWA, JAPAN—23 empty shells, north of Kohama Island, 24°21.5'N, 123°58.9'E, depth 15 to 20 m, crevices; 2 empty shells from "Sabachi Cave" diving site, Yonaguni Island, totally dark. DAITO ISLANDS (BORODINO ISLANDS), OKINAWA, JAPAN—2 empty shells from "Gon-gon-ana Cave" diving site, cavern, Minami-Daito Island, 25°50.68'N, 131°01.85'E, depth 23 m, gloomy. OGASAWARA ISLANDS (BONIN ISLANDS), JAPAN—1 empty shell from cavern, Otoutojima, gloomy; 2 empty shells from "Giant Cave" diving site, cave, Tatejima, totally dark. TINIAN—200+ empty shells from sta. TN1, huge cave close to "Tinian Grotto" diving site, 15°01.1'N, 145°35.0'E, depth 50 to 51 m, gloomy. SAIPAN—26 empty shells from "Grotto" diving site, cave, Saipan, 15°15.3'N, 145°49.6'E, depth 20 m, totally dark. GUAM—1 empty shell from cavern, Apra Point, 13°27'N, 144°37'E, depth less than 10 m, gloomy. PALAU—100+ empty shells from "Virgin Hole 1," cave branched from main tunnel in reef lagoon, 07°07.2'N, 134°14.1'E, depth 17 m, totally dark; 5 empty shells from "Siaes Tunnel" diving site, huge tunnel, 07°18.7'N, 134°13.6'E, depth 24 to 44 m, gloomy; 55 empty shells from "Blue Hole" diving site, huge cave, 07°08.3'N, 134°13.3'E, depth 36 m to 38 m, totally dark. POHNPEI—1 empty shell from sta. PO1, "Tawag Point" diving site, cavern, 06°53.0'N, 158°06.0'E, depth 26 m; gloomy. NAURU—7 empty shells from sta. NR1, cavern, AW Aiwo, 0°32.5'S, 166°54.5'E, depth 15 to 25.5 m, gloomy. NEW CALEDONIA—3 empty shells from sta. Pins 2, cavern, east of Nuw Powa, Iles des Pins, 22°31.6'S, 169°25.9'E, depth 17 m to 20.9 m, gloomy. VANUATU—6 empty shells from "Taj Mahal" diving site, cave, west of Efate Island, 17°38.4'S, 168°08.7'E, depth 15 m to 18 m, gloomy to totally dark. FIJI—1 empty shell from

a cavern, north of Ono Island, Great Astrolabe Reef, 18°51.8'S, 178°27.0'W, depth 7 m, gloomy inside. TONGA—15 empty shells from sta. VV3-13, "Swallows Cave" diving site, cave, southwest of Falevai Island, Vava'u Group, 18°40.9'S, 174°02.9'W, depth 17 to 18 m, gloomy; 3 empty shells from sta. HA-4, cavern, west of Haano Island, Ha'apai Group, 19°43.1'S, 174°17.4'W, depth 5 m, gloomy. TAHITI—72 empty shells from sta. TH1, TH1-4, "Cave Arue" diving site, cave, west of Tahiti, 17°30.9'S, 149°32.1'W, depth 22 m to 30 m, gloomy.

**Description:** Shell minute in size, up to 1.34 mm in width, depressed-turbiniiform, umbilicate, thin, fragile, opaque white in shell color, with height about  $\frac{3}{4}$  of width. Periostracum very thin, colorless. Protoconch smooth, 142 to 176  $\mu$ m in diameter, tip narrowly rounded, and separated from teleoconch by slightly flared rim. Teleoconch just two volutions in largest specimen, rapidly expanded, evenly rounded and separated by impressed suture; end of mature body whorl descending obliquely and steeply. Sculpture of first half whorl fine, sharp, regularly spaced collabral axial riblets, thereafter finely reticulating collabral riblets and fine spiral cords; intersection of both forming tiny tubercles. Umbilicus wide and deep, rim rounded, and sculptured only by axials inside. Aperture large, subovate, steeply prosocline, and not holostomous. Outer lip thin and sharp, parietal area narrow, and inner lip simply concave, thin, and sharp.

Operculum rudimentary, multispiral, thin, corneous, with diameter of  $\frac{1}{4}$  of shell aperture (Figure 3B).

Radula rhipidoglossate, consisting of approx. 85 transverse rows, with formula  $\infty + 5 + 1 + 5 + \infty$ . Central tooth broadest, enlarging toward base, cutting edge broad and straight, with nine cusps, median cusp largest. Laterals of one to four similarly shaped teeth, tightly interlocked outwardly near base; lateral 1 broader than laterals 2 to 4, cutting area with six cusps, second to innermost cusp longest; lateral 2 with four cusps, also second to innermost cusp longest; laterals 3 and 4 with five cusps, with longest cusp at center; shaft of lateral 5 becomes broader toward base with two angulations projected inwardly at middle and base, cusp six, fourth to innermost cusp longest, two outer teeth minute. Marginal field of more than 40 teeth in each side of row; teeth very slender, cutting edge subtriangular; innermost tooth with six long cusps.

Animal with long cephalic tentacles, closed eyes with short stalks, right subocular peduncle, long epipodial tentacles, right and left shell muscles and left ctenidium. Cephalic tentacles and peduncle bear micropapillae. Epipodial tentacles three on each side, all similar in length. Right shell muscle far larger than left. Left ctenidium monopectinate, with up to 11 leaflets; right ctenidium absent. Rectum terminating as anus near right shell muscle. Sexes separate; no size difference between male and female; brood pouch absent.

**Measurements:** Range of diameter of 30 specimens from the Philippines, Okinawa, and Palau, 0.87–1.34 mm (mean 1.06, SD 0.11); range of height (30 specimens), 0.64–0.94 mm (mean 0.79, SD 0.08); range of height/diameter ratio (30 specimens), 0.60–0.87 (mean 0.75, SD 0.06); range of protoconch diameter of 33 specimens from Philippines, Okinawa and Palau, 142  $\mu$ m–176  $\mu$ m (mean 157, SD 0.8).

**Remarks:** In teleoconch shape and surface sculpture this species resembles the species of the genus *Laroechea*. In addition to the lack of internal inner lip septum, *T. ohashii* differs from *Laroechea miranda* Finlay, 1927, *Laroechea secunda* Powell, 1937, and *Laroechea scitula* Marshall, 1993, in having a wide umbilicus and a small smooth protoconch (Figures 1A–C, 2A, B). *T. ohashii* is easily distinguished from *Laroecheopsis amplexa* Marshall, 1993, primarily by the reticulate surface sculpture and presence of a wide umbilicus. The new species is distinguished from *T. marshalli* primarily in having a broadly open umbilicus and coarser ornamentation. Both species have a similar meshlike ornamentation over the shell surface, but the intersections of the axial and spiral cords are spiny in *T. ohashii*, whereas they are not in *T. marshalli*.

**Etymology:** The species is named after Mr. Shu-ichi Ohashi, a professional SCUBA diver from Naha, Okinawa, Japan, who helped the authors in collecting the material.

*Troглоconcha tessellata* Kase & Kano, sp. nov.

(Figures 1D–F, 2C, D)

**Type specimens:** Holotype, NSMT Mo72830, 1.02 mm high, 1.11 mm wide; 7 paratypes, NSMT Mo72831.

**Type locality:** North of Kohama Island, Yaeyama Group, Okinawa (24°21.5'N, 123°58.9'E); depth 15 to 20 m; crevices; coral sand.

**Distribution:** Okinawa, Japan; only known from the type locality.

**Description:** Shell minute in size, naticiform, 1.11 mm in width in largest specimen, anomphalous, thin, fragile, opaque white in shell color, with width slightly greater than height. Spire elevated very slightly from body whorl. Periostracum unknown. Protoconch almost planispiral, smooth except for indistinct granules, about 0.16 mm in diameter, 1.25 in volution, tip narrowly rounded, and separated from teleoconch by slightly flared rim. Teleoconch just two volutions in largest specimen, rapidly expanded, evenly rounded, and separated by weakly impressed suture; end of mature body whorl descending obliquely and steeply. Sculpture of first half of teleoconch whorl prosocline, roundly curved, regularly spaced, sharp, 25 col-labral axial riblets; after ½ volution teleoconch whorl

starting to bear spiral cords finer than axial riblets, first on middle of upper surface and adding successively on both sides of first one, finely granulated at intersections of axial riblets. Body whorl round-sided, sculptured with dense and very fine, prosocline axial and spiral cords. Aperture large, subcircular, moderately prosocline and interrupted at base of previous whorl. Outer lip thin and sharp, parietal area weakly convex, and inner lip slightly reflected at abaxial end. Soft part unknown.

**Measurements:** Range of shell diameter of eight specimens, including holotype, 0.75–1.14 mm (mean 0.91, SD 0.11); height, 0.69–1.02 mm (mean 0.83, SD 0.10); height/diameter ratio, 0.90–0.96 (mean 0.91, SD 0.02).

**Remarks:** This species is represented only by eight empty shells and is known only from the type locality, where it is associated with *T. ohashii*. It has a smooth protoconch, and sculpture pattern in the early teleoconch whorl and apertural features almost identical to *T. ohashii*. *T. tessellata* has a similar shell form and protoconch morphology to those of *Laroecheopsis amplexa*, but the meshlike sculpture pattern over the teleoconch surface of the new species differs from the shallow pits in the early whorls and spiral threads in the later whorls of the latter species. The allocation of this species to *Troглоconcha* gen. nov. is based on the greater similarity of the shell to *T. ohashii* rather than to *L. amplexa*.

*Troглоconcha tessellata* most closely resembles *Troглоconcha marshalli* from the upper Oligocene of France in its overall shell characters, strongly suggesting congeners for both species. The new species is only separable from *T. marshalli* in the absence of an umbilicus. It is also distinguishable from *T. ohashii* in its higher proportion of shell form, less steeply inclined outer lip, much finer meshlike ornamentation, and lack of open umbilicus.

**Etymology:** From the Latin, *tesselatus* (adv.), meaning tessellated with reference to the finely reticulate ornamentation of the whorls.

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## Reproductive Cycle of the Bivalves *Ensis macha* (Molina, 1782) (Solenidae), *Tagelus dombeii* (Lamarck, 1818) (Solecurtidae), and *Mulinia edulis* (King, 1831) (Mactridae) in Southern Chile

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**Abstract.** The reproductive cycles of the bivalves *Ensis macha* (Molina, 1782), *Tagelus dombeii* (Lamarck, 1818), and *Mulinia edulis* (King, 1831) were studied at six sites in southern Chile (38–43°S) from November 1996 to December 1997. Samples of *E. macha* came from three subtidal shallow depths; those of *T. dombeii* from two subtidal depths and one intertidal site; and samples of *M. edulis* originated in one subtidal shallow depth and one intertidal site. Thirty specimens were collected monthly for standard histological analyses. Water samples were also collected to determine salinity, temperature, and chlorophyll *a* content. In general, the reproductive cycles of the three species were characterized by long spawning periods, beginning during late spring-summer. In some cases, that period extended during autumn-winter until the following spring. The gonads of most of the populations showed quite short recovery periods, with the exception of populations located farther south, which needed more time to begin a new cycle. Comparison of subtidal versus intertidal populations showed that the gonad stages developed more slowly for the latter populations. The earlier results show that variability exists in the timing of gametogenic cycles of *E. macha*, *T. dombeii*, and *M. edulis* along the coast of southern Chile. No significant relationship was found between seasonal variability of reproductive stages and seasonal variability of water characteristics. Among these characteristics, water temperature and chlorophyll *a* content were the most important. Potential fecundity varied geographically in *E. macha* and *T. dombeii*, whereas, in general, no variability was observed in mean sizes of oocytes of the three species. These results must be taken into account when management plans are designed; thus, the timing of the gametogenic cycles of bivalves of economic importance must be studied along their full geographic ranges.

### INTRODUCTION

Knowledge of the reproductive cycles of marine invertebrates of economic importance is basic to culture activities and management of natural stocks. In that way, it is possible to regulate fishery activities and set up closing seasons to preserve the species, via the protection of reproductive individuals (e.g., Defeo 1987, 1989, 1993).

The reproductive cycles of different bivalve species are unique for each population, varying according to geographic location (Sastry, 1979). The high seasonality of the environment in medium to high latitude locations results in annual cycles, with gametogenesis during winter, and spawning during the spring-summer season. In contrast, the reproductive cycle in low latitude locations is characterized by long or continuous spawning periods (Heffernan & Walker, 1989; Heffernan et al., 1989a, b). Environmental factors such as water temperature and salinity, photoperiod, and food resource availability have been mentioned as concrete causes for that latitudinal variability (Giese & Pierse, 1977; Mackie, 1984). The latitudinal variability in the reproductive cycle of bivalves

has been particularly studied in species of economic importance (Tarifeño, 1980; Manzi et al., 1985; Heffernan et al., 1989a, b; Laasuy & Simons, 1989; Kanti et al., 1993; Urban & Campos, 1994; Villalejo-Fuerte et al., 1996a; Gallardo & Weber, 1996).

Apart from the variability in reproductive cycles related to geographic variation, it has been shown that zonation across shore also influences some of the characteristics of the reproductive cycles such as the production of somatic and sexual tissue and differences in fecundity and size of oocytes (McLachlan, 1974; Griffiths, 1981; Harvey & Vincent, 1989, 1991; Richardson, 1993; Walker & Heffernan, 1994; Brousseau, 1995). For example, Borrero (1987) found three important differences between subtidal and intertidal populations of the mytilid *Geukensia demissa* (Dillwyn) in South Carolina, USA: time of the onset of gametogenesis, time of occurrence of spawning, and length of time remaining in a mature reproductive condition before spawning. That was probably due to changing conditions in submergence throughout the tidal cycle, which result in changing environmental tem-

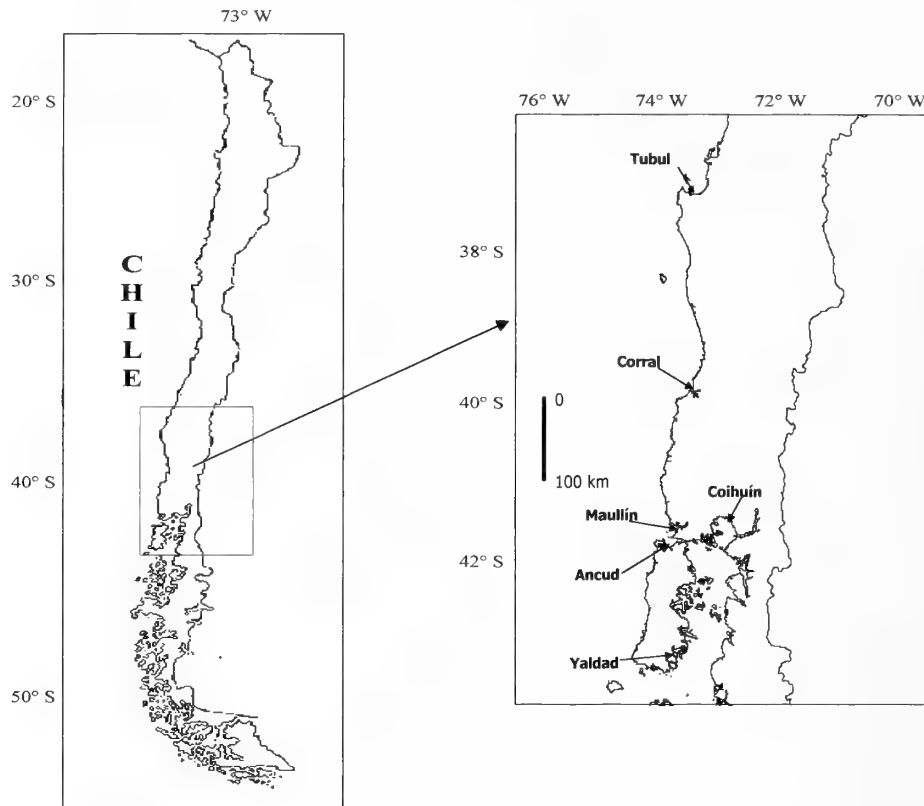


Figure 1. Location of sampling sites at the coast of south central Chile.

peratures and time for feeding, conditions that may influence the reproductive output of invertebrates (Barber & Blake, 1981; Bayne & Newell, 1983).

The coast of southern Chile (38–43°S) is characterized by small bays and numerous microtidal estuaries. Some of the most common bivalves are *Ensis macha* (Molina, 1782) (Solenidae); *Tagelus dombeii* (Lamarck, 1818) (Solecurtidae); and *Mulinia edulis* (King, 1831) (Mactridae). *Ensis macha*, *Tagelus dombeii*, and *Mulinia edulis* occur along a wide latitudinal range of the Chilean coast, the first species from Caldera (approx. 27°S) to Magallanes (55°S), and the latter two species throughout all the Chilean coast (Osorio et al., 1979). They are among the most common bivalves subjected to commercial fisheries along the coast of southern Chile (approx. 38–43°S). Approximate commercial sizes are 100–180 mm for *E. macha*, 70–90 mm for *T. dombeii*, and 50–70 mm for *M. edulis*. Landing fisheries along the Chilean coast started in 1988 for *E. macha*, 1965 for *T. dombeii*, and 1994 for *M. edulis*.

In recent years, landing figures for *E. macha*, *T. dombeii*, and *M. edulis* in southern Chile (about 38–43°S, Arauco to Quellón, Figure 1) represented an average of 99.7, 99.5, and 80.7% of the total national landing figures (data from 1988–1997 for *E. macha* and *T. dombeii*, and from 1994 to 1997 for *M. edulis*). During the period

1988–1997, the highest landing figures from *E. macha* and *T. dombeii* were 8595 (1991) and 7260 annual tons (1988), respectively. From 1994–1997, the maximum landing for *M. edulis* reached 2553 annual tons (1994). The vast majority (approx. 90%) of landings are used in the canning industry (Sernapesca, 1998).

Despite the economic importance of these three species, there are few studies which deal with the effect of geographic variability on their reproductive biology. Santos-Salas et al. (1998) and Aracena et al. (1998) described growth and feeding of juveniles and the reproductive cycle of *E. macha* in shallow waters of Golfo de Arauco (approx. 38°S). For *M. edulis* there is only one study on the external morphology of larvae (Fuentes, 1988), and another study on production of spats (Paredes & Hernández, 1986). For *T. dombeii* there are several studies on its reproductive cycle at several localities on the Chilean coast, showing variations in its reproductive cycle (Lasen, 1979; Fierro, 1981; Arratia, 1998).

Because of the variability of coastal waters along the Chilean coast (Brattström & Johanssen, 1983; Strub et al., 1998; Viviani, 1979), it is reasonable to expect some variability in reproductive cycles along the latitudinal ranges of these species. The purpose of the present study was to analyze the reproductive biology of *E. macha*, *T. dombeii*, and *M. edulis*, (Figure 2) at different areas of a coastal

range spanning approximately 600 km of the southern Chilean coast. Because *E. macha* lives primarily under water, we obtained only subtidal samples for this species. For *T. dombeii* and *M. edulis*, however, we obtained intertidal and subtidal samples. Thus, for *T. dombeii* and *M. edulis* we were able to analyze populations living at different latitudes and depths.

## MATERIALS AND METHODS

### Study Area

Samples were collected from six sites on the coast of southern Chile (Figure 1). Subtidal samples of *E. macha* were collected from Tubul, Golfo de Arauco (37°14'S, 73°29'W), Bahía de Corral (Corral hereafter) (39°50'S, 73°28'W), and Bahía de Ancud (Ancud hereafter) (41°50'S, 73°47'W). Subtidal samples of *T. dombeii* came from Tubul and Corral; intertidal samples of this species were collected from Coihuin, Golfo del Reloncaví (41°28'S, 72°41'W). Subtidal samples of *M. edulis* were collected at Maullín (41°39'S, 73°37'W); intertidal samples of this species came from Yaldad (43°07'S, 73°44'W) (Figure 1).

### Sampling and Treatment of Samples

Samples were collected from November 1996 to December 1997. Subtidal samples were collected by semi-autonomous diving from shallow water beds (7–14 m depth). Intertidal samples were collected during spring low tides. Due to rough sea conditions, no samples were collected during April in Corral and during May at Tubul and Corral.

For histological analysis of the gonads, samples of 30 specimens were collected from each one of the study sites. The bivalves were kept at low temperature (3°C) to be processed within 24 hours after collection. After dissection, the gonads were fixed in aqueous Bouin's fixative. After embedding in paraffin, 7 µm serial sections were cut and stained with hematoxylin and eosin (Bancroft & Stevens, 1977). Ten sections of the gonad of each specimen were examined under the light microscope to determine the gonadal organization and the seasonal gametogenic cycle. The following categories of gonad development were used in this study (cf. Peredo et al., 1987; Brousseau, 1995).

**Early Active.** Phase of gamete proliferation and development. Gonadal follicles are small and have thick walls; the interstitial tissue is abundant and disseminated among the gonadal follicles. In males, spermatogonia are close to the follicular walls, while few spermatids and spermatocytes are located near the center of the follicles. In females, oogonia can be seen embedded in the follicular walls; pre-vitellogenic oocytes and vitellogenic oocytes with cytoplasm extend into the lumen of the follicles.

**Late Active.** This is the phase of gamete maturation. In

both sexes a reduction of gonias and an increase in the mature gametes (oocytes and spermatozoa) can be seen. In males the sperm form radially oriented columns with their tails toward the center of the follicles. In the female gonad, vitellogenic oocytes are more numerous, and some mature oocytes are free in the lumen of the follicles.

**Ripe.** Gonadal follicles are expanded in these stage with their walls being very thin and with a lower number of early stage cells. In males, mature sperm form dense masses and cells. In females, the follicles are crowded together and filled with mature oocytes.

**Partially Spawning.** In both sexes the follicles still contain gametes, but these are less numerous than in the ripe stage. It is still possible to see gametes in early stage (spermatids and vitellogenic oocytes attached to the wall).

**Spent.** In both sexes, most of the follicles are devoid of gametes with some residual mature spermatozoa or oocytes.

**Recovery.** Most of the follicles are devoid of gametes, although some follicles have a few residual gametes. The interstitial tissue has increased and surrounded the follicles.

The stereometric technique of Weibel (1969) was used for fecundity determination, i.e., the volume of different cellular components was determined from the gonad analyses, through the relationship between the surface of that component and the total surface (Neuer, 1966). The diameter of 100 oocytes (from different females) was measured, using an eyepiece graticule calibrated with a stage micrometer. Measurements were made along the longest and the shortest axis of the oocytes. From these data, mean oocyte size and standard deviation were obtained. Maturity of the oocytes was determined according to Peredo et al. (1987) and Masello (1987), i.e., free oocyte in the light of the follicle with cytoplasm of the lumpy aspect and acidophile; large rounded nucleus; clearer color than cytoplasm with a very clear nuclear membrane and granulated chromatin; large nucleoli in the interior to which small ones can be added.

Water samples from the subtidal sampling sites (Tubul, Corral, Maullín, and Ancud) were collected from about 50 cm above the bottom to determine temperature, salinity, and chlorophyll *a* content. At Coihuin and Yaldad (intertidal sites) water samples were collected during rising tides (about 50 cm depth). Temperatures were measured *in situ* with a mercury thermometer ( $\pm 0.1^\circ\text{C}$ ). Salinity was measured with a portable salinometer Hydro-bios. The chlorophyll *a* content was measured after the filtration of 2 liters of water in Milipore filters with 0.45 µm of opening. The filters were kept at low temperatures ( $-7^\circ\text{C}$ ) and in darkness. After a short period (5–7 days), they were kept in 90% acetone for 24 hours to extract pigments, and centrifuged at 3500 rpm for 15 min. The absorbance of the supernatant was measured at 750 and 665 nm (Strickland & Parsons, 1972).

One-way analysis of variance (Sokal & Rohlf, 1995)

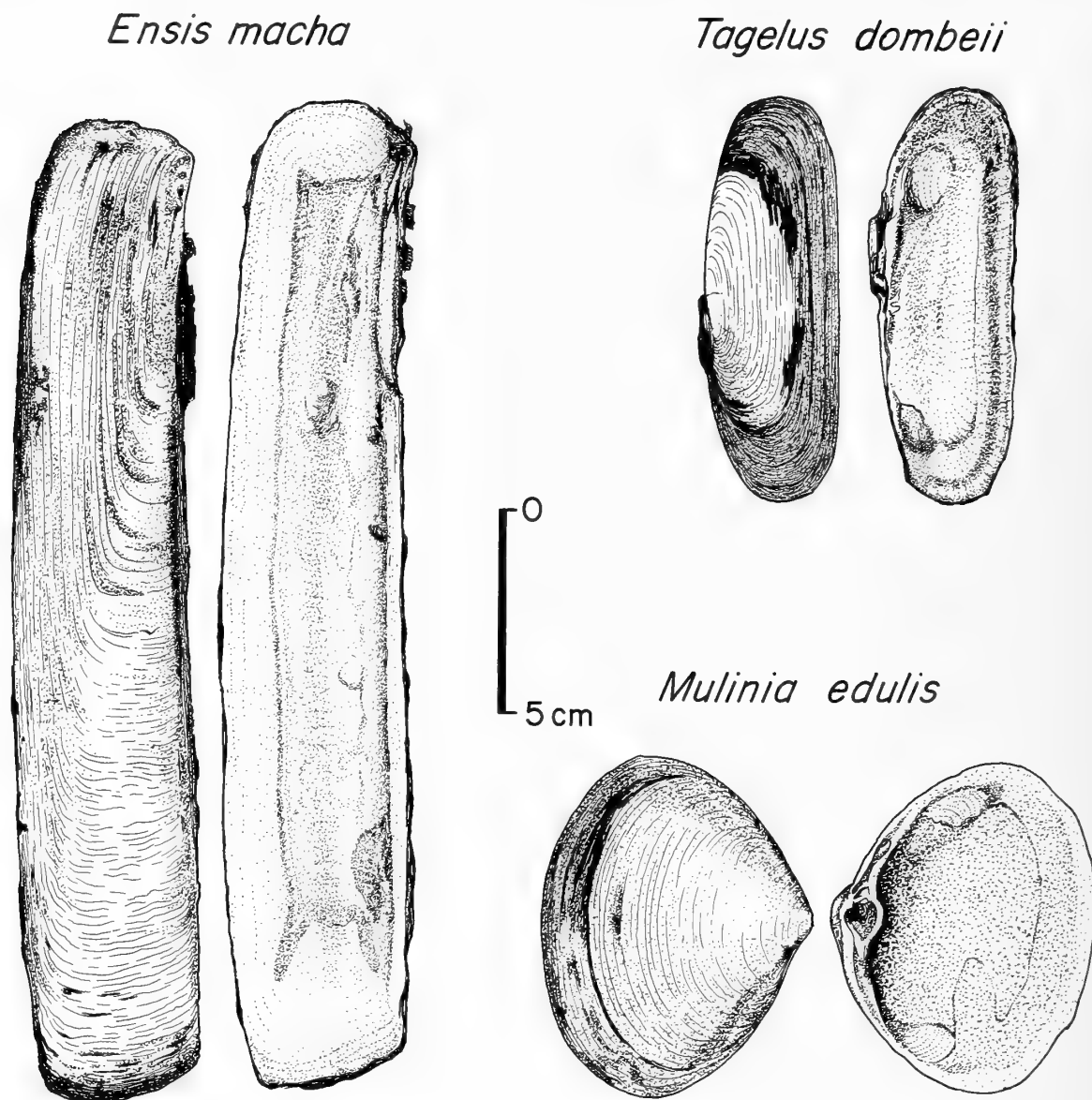


Figure 2. External and internal views of shells of *Ensis macha*, *Tagelus dombeii*, and *Mulinia edulis*.

was used to compare the mean potential fecundity among bivalves of the same species collected at different sites. If the analysis of variance indicated significant differences among means ( $P < 0.05$ ), these were compared using the *a posteriori* Tukey's multiple comparison test (Day & Quinn, 1989). Due to the fact that mean oocyte diameter did not have a normal distribution, a non-parametric analysis (Kruskal-Wallis ANOVA) was used. Simple regression analysis (Sokal & Rohlf, 1995) was carried out to evaluate relationships between percentages of mature (ripe stage) and spawned (partially spawned and spent stage) and variability of water temperature and chlorophyll *a* water content.

## RESULTS

### Water Characteristics

Figure 3 shows the temporal variability in temperature, salinity, and chlorophyll *a* content of water at each study site. Water temperature showed small variability at Tubul: from 12°C in March to 14.5°C in April and July. More seasonal variability was found farther south; from a minimum of 11, 10, 10, 12 and 10°C during winter time to a maximum of 13.6, 16, 18.5, 16, and 15°C during late spring-summer at the waters of Corral, Maullín, Coihufín, Ancud, and Yaldad, respectively (Figure 3). Water salinity varied little at the shallow waters of the bays of Tubul,

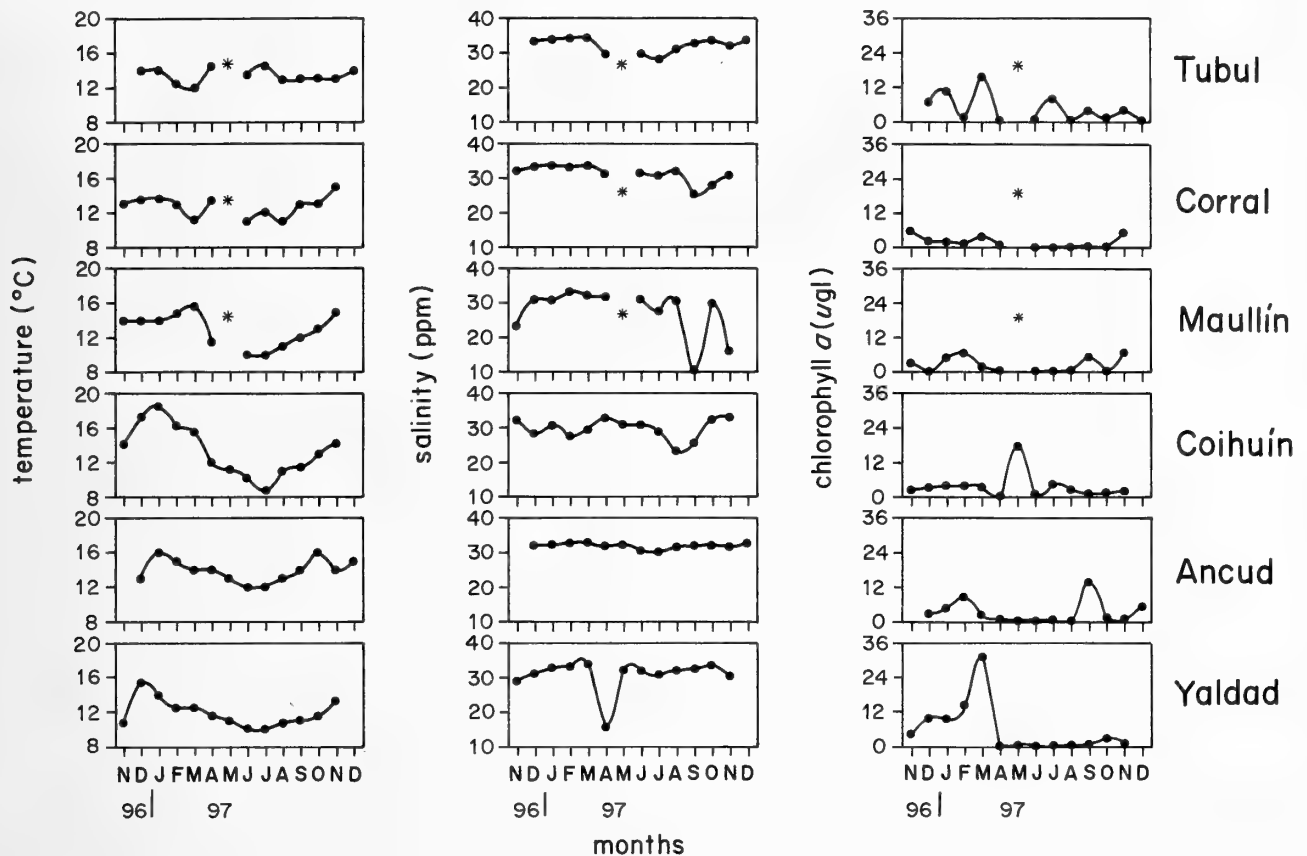


Figure 3. Temporal variability of temperature, salinity, and chlorophyll *a* content in the waters of the sampling sites. \* = no data

Corral, and Ancud (from about 28–34 ppm). More variability was found at the shallow waters of Maullín (10–33 ppm), and at the intertidal sites of Coihuín and Yaldad (15–34 ppm). In general, the highest content of chlorophyll *a* at all study sites was found during summer (December–February) and spring months (September–November), whereas the lowest occurred during late fall and winter. During late summer of 1997 (March), the intertidal waters of Yaldad registered the highest chlorophyll *a* value found in this study (31.2 µg/l) (Figure 3).

### Morphology of Gonads

Microscopic analyses revealed that *E. macha*, *T. dombeii*, and *M. edulis* are dioecious species without external sexual dimorphism. While no color differences exist in the gonads of *E. macha* and *T. dombeii*, the gonads of *M. edulis* vary in color, being dark royal purple in females, and orange in males. The gonad structure is similar in the three species; it is embedded in the visceral mass together with the hepatopancreas and gut. It does not have any kind of enveloping sheet; in some regions it is dissected by muscular strings originating in the body wall.

### Gametogenic Cycles

*Ensis macha*. Figure 4 shows the frequency of the different gonad stages of *E. macha* at the shallow waters of Tubul, Corral, and Ancud. At the beginning of the study (November–December 1996), the three populations were in late active and ripe stages. During January, 23% and 13% of the individuals collected at Corral and Ancud had partially spawned. Twenty-seven percent of the animals collected during February in Tubul had reached this stage. During late summer (March), 100%, 93%, and 50% of the populations of Tubul, Corral, and Ancud, respectively, were partially spawned. One hundred percent of the animals of Tubul and Ancud were in this stage during April. During the winter and spring months, the three populations showed significant differences in their gametogenic cycles. During June and July, about 55% of the specimens of *E. macha* in Tubul had their gonads in early and late active stages. From June to August, percentages of ripe individuals varied (approx. 20–50%). During August, partially spawned individuals were collected again (approx. 40%); this last stage persisted during the rest of the spring (September–December). During June–August,

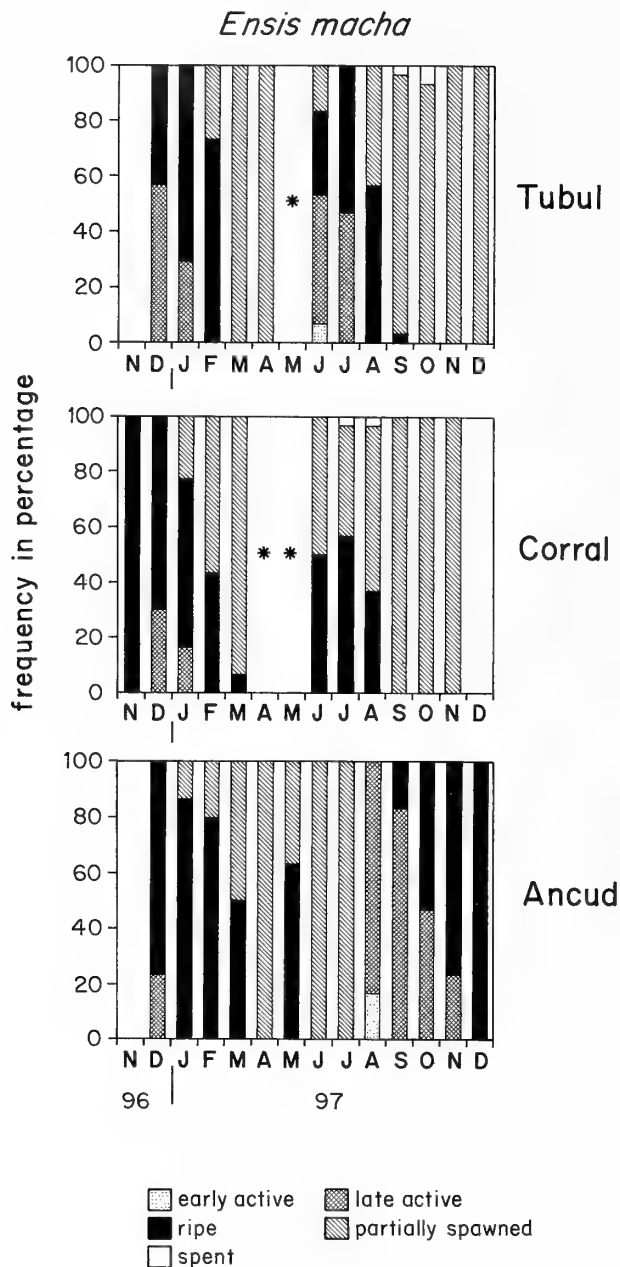


Figure 4. Seasonal variability in the frequencies (percentages) of the different stages of the gametogenic cycle of the gonad of *Ensis macha* at the shallow subtidal of Tubul, Corral, and Ancud. \* = no data

the population of Corral was represented by ripe and spawned animals in similar proportions. From September to November, 100% of the animals were in partially spawned stage. The specimens collected during May in Ancud were ripe and partially spawned; during June–July, 100% of them were in this last stage. From August to November, individuals were either recovering (late active

stage) or mature (ripe stage). During December, 100% of the animals collected in Ancud were mature.

*Tagelus dombeii*. Figure 5 shows the sequence of the reproductive cycle of *T. dombeii* at the shallow waters of Tubul and Corral and at the intertidal of Coihuin. During the first 2 months of the study (November and December 1996), the three populations were in different reproductive stages. Whereas the animals of Tubul were in late active or ripe stage, those of Corral and Coihuin were ripe or spawned (primarily at Corral, 97% during December). During January, the individuals of Tubul had yet not spawned, whereas most of the individuals from Corral and Coihuin were spawning, with some specimens also in the ripe stage (5% and 20%, respectively). The beginning of a new cycle (individuals in early active stage) was observed in June for the individuals of Tubul and Coihuin, and in July for the specimens of Corral. During late winter (August–September), the population of Tubul was spawning, even when ripe animals were also collected. At the same time, the populations of Corral and Coihuin were still in early and late active stages. During the spring (October–December), most of the specimens collected at Tubul were spawning. All the individuals collected at Corral were in late active stage during October, and ripe during November. In Coihuin, most of the individuals (approx. 95%) were ripe in October; and in November, half of the population was partially spawned.

*Mulinia edulis*. The reproductive cycle of *M. edulis* at the shallow waters of Maullín and at the intertidal of Yaldad is shown in Figure 6. During November 1996, different reproductive stages were found (early active, late active, and ripe stage) at both sites. During summer (December–March), both populations were in ripe and partially spawned stages, with the highest percentage of spawned individuals found at the end of the summer (approx. 50% in Maullín and 77% in Yaldad). Both populations were in similar stages during April to June. Specimens collected at Maullín during July were in recovery, ripe, and spawn stages, quite a similar situation (but without recovery stages) to that observed for the intertidal population of Coihuin. During August, both populations were in similar stages of the gametogenic cycle. From September to November, the specimens of Maullín were spawning, while those of Yaldad had begun a new cycle (early and late active stages), an assertion supported by the dominance of ripe stages during November.

#### Gametogenic Cycles and Water Characteristics

Table 1 shows the results of regression analyses carried out between the seasonal variability in the percentages of mature (ripe stages) and spawned individuals (partially spent and spawned) and the temporal variability of water characteristics. The temporal variability in the percentage of mature females of *E. macha* at Tubul was positively correlated with that of chlorophyll *a* content. The tem-

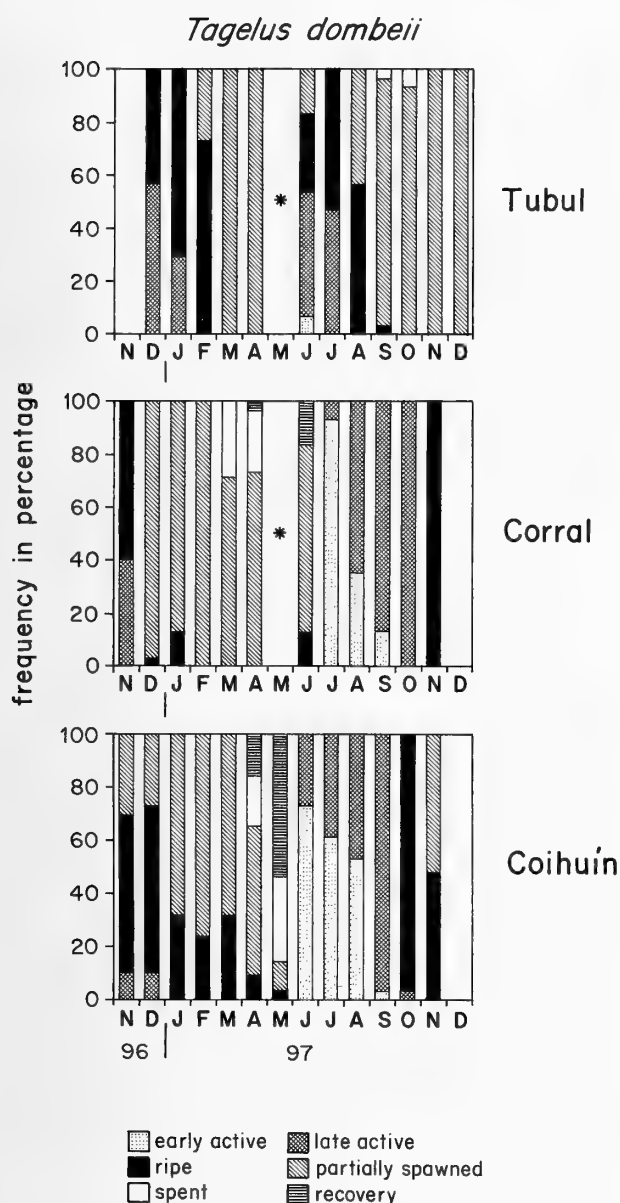


Figure 5. Seasonal variability in the frequencies (percentages) of the different stages of the gametogenic cycle of the gonad of *Tagelus dombeii* at the shallow subtidal of Tubul and Corral and the intertidal site of Coihuín. \* = no data

poral variability in the percentages of spawned specimens (either the whole population or males and females by themselves) was also correlated with the chlorophyll *a* content, but inversely. Only the females of *E. macha* showed a significant relationship to the water characteristics at Corral; mature females were more abundant when chlorophyll *a* was higher, whereas spawned females peaked when chlorophyll *a* was lower. The results found from *E. macha* at Ancud showed that mature individuals were more abundant when temperatures were higher,

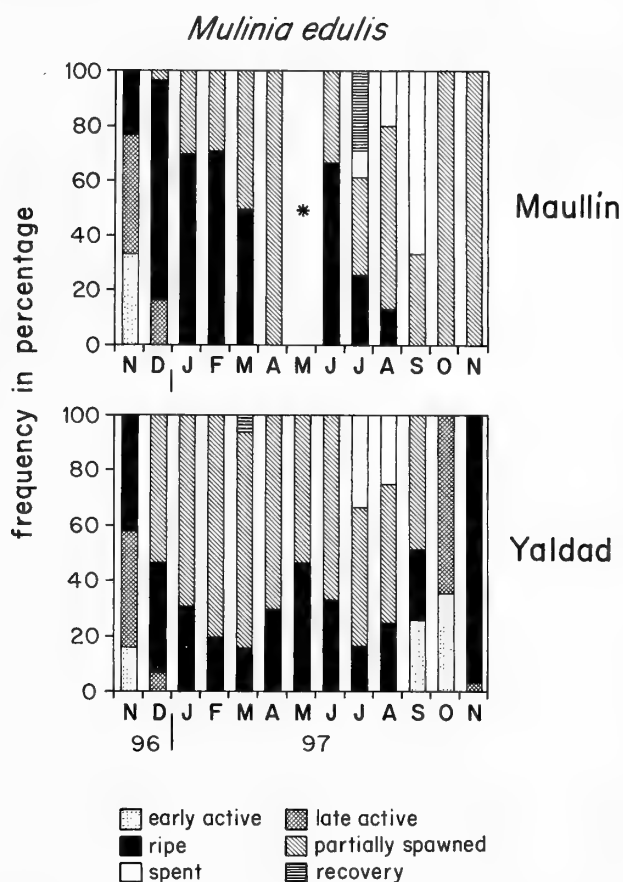


Figure 6. Seasonal variability in the frequencies (percentages) of the different stages of the gametogenic cycle of the gonad of *Mulina edulis* at the shallow subtidal of Maullín and the intertidal site of Yaldad. \* = no data

whereas spawned females peaked when temperatures were lower. At the intertidal site of Coihuín, a significant correlation was found between the temporal variability of the reproductive stages of *T. dombeii* and physical factors; thus, percentages of total individual spent, mature females and spawned individuals were more abundant when water temperatures were higher. Similarly, percentage of mature females of *M. edulis* at the intertidal site of Yaldad peaked when water temperatures were higher (Table 1).

#### Diameter of Oocytes and Potential Fecundity

The mean diameter of oocytes was 50–51  $\mu\text{m}$  for *E. macha*, 38–39  $\mu\text{m}$  for *T. dombeii*, and 41–42  $\mu\text{m}$  for *M. edulis*, without significant differences between sites ( $P > 0.05$ , Table 2). Mean potential fecundities were higher for *E. macha*, particularly in Tubul and Corral where those fecundities (approx.  $18\text{--}19 \times 10^6$  oocytes per individual) were significantly higher ( $p < 0.05$ ) than that estimated for the population sampled at Ancud (Table 2). The high-



Table 1

Results of the regression analyses carried out between the percentages of mature and spawned animals and the temporal variability of water temperature and chlorophyll *a* content at the study sites. Results are only given for those analyses which rendered significant correlations.

Species	Study site	Regression equation	r	p
<i>Ensis macha</i>	Tubul	% mature females = $-5.74 + 6.55 \text{ chlor } a$	0.88	0.00
		% spawned males and females = $83.28 - 5.65 \text{ chlor } a$	-0.58	0.04
		% spawned females = $86.59 - 6.06 \text{ chlor } a$	-0.59	0.04
	Corral	% spawned males = $724.96 - 49.79 \text{ chlor } a$	-0.81	0.00
		% mature females = $10.79 + 14.44 \text{ chlor } a$	0.69	0.02
		% spawned females = $89.27 - 16.04 \text{ chlor } a$	-0.71	0.01
	Ancud	% mature males and females = $-197.41 + 17.51^{\circ}\text{C}$	0.61	0.03
		% mature females = $-220.67 + 18.88^{\circ}\text{C}$	0.59	0.03
		% spawned females = $313.46 - 19.91^{\circ}\text{C}$	-0.58	0.04
<i>Tagelus dombeii</i>	Coihuín	% population spent = $-55.45 + 6.67^{\circ}\text{C}$	0.62	0.02
		% mature females = $-66.78 + 7.20^{\circ}\text{C}$	0.68	0.01
		% spawned females = $-62.53 + 6.66^{\circ}\text{C}$	0.68	0.01
		% spawned males = $-74.78 + 8.2^{\circ}\text{C}$	0.67	0.01
<i>Mulinia edulis</i>	Yaldad	% mature females = $-103.68 + 10.12^{\circ}\text{C}$	0.57	0.04

est mean potential fecundity for *T. dombeii* was estimated for the subtidal population sampled at Tubul. That fecundity (approx.  $11 \times 10^6$  oocytes per individual) was significantly higher than that estimated for the subtidal population of Corral ( $8 \times 10^6$  oocytes per individual) and the intertidal samples of Coihuín (approx.  $6 \times 10^6$  oocytes per individual), the last ones without significant differences between them (Table 2). The estimated mean potential fecundities estimated for the subtidal and intertidal populations of *M. edulis* (Maullín versus Yaldad) ranged from approx.  $10\text{--}14 \times 10^6$  oocytes per individual without significant differences between them (Table 2).

## DISCUSSION

The results of this study show that in the study area *E. macha*, *T. dombeii*, and *M. edulis* have an annual cycle of reproduction with periods of extensive spawns beginning during late spring-summer (November 1996 to February 1997). That period extends until autumn, and in some cases continues without stopping until next spring as was observed clearly in the intertidal population of *M. edulis* in Yaldad. In general, the recovery periods of the gonads are quite short and extend for more than 2 months just in the populations of *T. dombeii* in Corral and Coihuín, being the population studied of this species at the last place with the clearest annual cycle (i.e., all the gonad stages well represented).

The reproductive cycle of *E. macha* in Tubul and Corral was rather similar, with a quick recovery of the gonad during winter, after which spawning extended until the end of the study. The population of Ancud showed a more marked annual cycle with a new maturation period during the spring continuing toward the summer; spawning began during early summer (January 1997).

The reproductive cycle of *T. dombeii* of Tubul differed from that of Corral and Coihuín. The individuals collected from the first site began a new cycle during winter (June–July) which resulted in ripe individuals and spawning throughout spring. On the other hand, the beginning of a new cycle for the populations of Corral and Coihuín spanned a longer period of time; consequently, no spawning was found for Corral at the end of this study, whereas about 50% of the individuals of Coihuín were in this stage for that time. Thus, the gonads of the populations of *T. dombeii* located farther south take a longer time to go through all the stages before spawning.

The gametogenic cycle of *M. edulis* also showed intersite variability. While the subtidal population showed a continuous spawning and no beginning of a new cycle during the study period, the intertidal populations (also spawning almost all year round) showed the beginning of a new reproductive cycle during early spring.

The earlier results show a variability in the gametogenic cycles of *E. macha*, *T. dombeii*, and *M. edulis* along the geographic range studied.

Sastry (1979) considered water temperature as one of the most important factors in the regulation of the different stages of the reproductive cycle in marine invertebrates. Indeed, temperature is one of the main causes of differences in the timing of gametogenesis and spawning of different populations of the same species (Ropes, 1968; Tarifeño, 1980; Manzi et al., 1985; Urban & Campos, 1994; García-Domínguez et al., 1996). Vilalejo-Fuerte et al. (1996a) showed that in Baja California, Mexico, the increase in temperature inhibits gametogenesis in the cockle *Laevicardium elatum* (Sowerby). In other species of bivalves located in the Gulf of California, the spawning period is directly related to decrease of water tem-

Table 2

Mean size of oocytes and mean potential fecundity (with standard deviations in parentheses) for each species at the study sites. A summary of the statistical analyses (F and p; see Material and Methods) is also given. The same capital letters indicate no significant differences in the fecundity comparisons; different letters for the opposite results (i.e., significant differences).

Species	Study site	K-W ANOVA		Mean size of oocytes ( $\mu\text{m}$ )	ANOVA		
		F	p		F	p	
<i>Ensis macha</i>	Tubul	1.79	0.41	51 (5.1)	6.26	0.00	A
	Corral			50 (6.5)			A
<i>Tagelus dombeii</i>	Ancud	0.51	0.77	50 (6.5)	11.98	0.00	B
	Tubul			38 (4.6)			A
	Corral			39 (4.8)			B
	Coihuín			39 (5.1)			B
<i>Mulinia edulis</i>	Maulín	0.42	0.51	41 (4.7)	3.79	0.07	A
	Yaldad			42 (3.0)			A

perature (Villalejo-Fuerte et al., 1995). There are, however, other species in which the water temperature does not influence gametogenic cycles (García-Domínguez et al., 1996).

In this study, we found that water temperature affected in some way the timing of the gametogenic cycle of *E. macha* in Ancud, *T. dombeii* in Coihuín, and *M. edulis* in Yaldad. Ancud was the southern study site for *E. macha*, while Coihuín and Yaldad were the intertidal sites. It is indeed possible that at those places, a wider temperature variability occurred than that measured here, which may explain the above-mentioned relationship between timing of reproduction and water temperature.

Food resources are also important, and the percentage of individuals in different gonad stages is related to food amount and availability, primarily during the final period of maturation of the gametes (late active and ripe stages) and during the period of spawning (Sastry, 1979; Bayne & Newell, 1983; MacDonald & Thompson, 1985; Villalejo-Fuerte et al., 1996a). In this way, the females have enough energy to carry out the vitellogenic process, and later, larvae also have enough food for subsistence. The direct and significant relationship between the percentage of mature females of *E. macha* and *T. dombeii* and the concentration of chlorophyll *a* in the localities of Tubul and Corral suggest that food is the main cause of final maturation of the oocytes. The other populations could obtain that energy from that accumulated in their body or from another food type different to the phytoplankton (e.g., dissolved organic matter). For an intertidal population of *Semele solida* (Gray) in Coihuín, Arratia (1998) determined a continuous reproductive cycle, with liberation of gametes during the 15 months of the study, even during periods of low food concentration. Similar results were found by Jaramillo & Navarro (1995) for the mytilid *Aulacomya ater* in Yaldad. It has been demonstrated or suggested for bivalves that the transfer of nutrients exists from the somatic tissue toward the gonad during gametogenesis (Sastry & Venn, 1979; Barber & Blake, 1981; Villalejo-Fuerte et al., 1996b). Le Pennec et al. (1991) demonstrated for the pectinid *Pecten maximus* L. that other pathways exist for energy incorporation to the development of gametes, i.e., the recycling of atresic material and direct transfer of metabolites from the intestinal loop to the developing gametes. Sastry (1979) also mentioned the importance of dissolved organic matter, bacteria, and organic aggregates as a food source for bivalves, apart from the usual phytoplankton. Clasing et al. (1998) found at the intertidal flats of Coihuín that *Semele solida* spawns during autumn-winter when concentrations of phytoplankton are low. These authors stated that because *Semele solida* is able to use the organic matter deposited on the surface sediments that organic matter would be an alternate energy source during autumn and winter. A similar explanation has been also given for the continuous

reproductive cycle of the intertidal bivalve *Diplodonta inconspicua* at the same site (Clasing et al. 1998).

The comparison between gametogenic cycles of subtidal versus intertidal populations shows that intertidal populations exhibit a slower development of the different stages of the reproductive cycle. Landers (1954) suggested that intertidal individuals of *Mercenaria mercenaria* living in the east coast of USA spawn earlier than subtidal individuals. Borrero (1987) also found that the beginning of gametogenesis and spawning in *Geukensia demissa* (Dillwyn) occurs earlier in low intertidal populations than high intertidal populations. Walker & Heffernan (1994) showed that the reproductive pattern of populations of *Mercenaria mercenaria* of Georgia (USA) is affected by the emersion time. On the other hand, Eversole et al. (1980) did not find differences in the reproductive parameters of subtidal and intertidal populations of the same species in the coast of South Carolina. Brousseau (1995) compared the results obtained for intertidal populations of *Crassostrea virginica* (Gmelin) in Long Island (USA) with those obtained before by Loosanoff (1942) 40 years earlier for subtidal populations, and concluded that not much difference exists as far as timing of the reproductive cycle is concerned.

The comparison of potential fecundity showed that the values estimated for *E. macha* were higher than those for *T. dombeii* and *M. edulis*. These differences may well be related to volume differences in gonadal tissue among species, a parameter directly correlated to the body size of individuals, i.e., the larger *E. macha* probably has larger gonads. Although no estimation of volume of gonads was carried out in this study, the body length of *E. macha* is longer than that of the other two species (see Figure 2). The potential fecundity of *E. macha* and *M. edulis* studied here is similar to other species of Chilean bivalves with plantotrophic larvae such as *Aulacomya ater* (Molina), *Choromytilus chorus* (Molina), *Venus antiqua* (King & Broderip), *Eurhomalea rufa* (Lamarck), *Mesodesma donacium* (Lamarck), and *Prothothaca thaca* (Molina) (Lozada, 1989). The results of this study showed that for *E. macha* and *T. dombeii* the potential fecundity was lower farther south. Differences in body size among sites may also be invoked to explain that result, i.e., the larger females collected farther north (Jaramillo et al., 1998, unpublished data) had larger gonads and thus, more potential fecundity.

However, the eventual effect of body size on size of gonads and potential fecundity seems to not affect mean sizes of oocytes. As a matter of fact, our results did not show any consistency. Thus, subtidal and intertidal populations of *T. dombeii* and *M. edulis* did not show significant differences in mean sizes of oocytes. This is different to the findings of other authors, such as Walker & Hefferman (1994) who found in England that subtidal populations of *Mercenaria mercenaria* have larger gonads and more oocytes than specimens living higher up

the coast. Also, Harvey & Vincent (1989, 1991) found that differences in exposure time to air during low tide is coincident with differences in potential fecundity of *Macoma balthica* inhabiting tidal flats of the Saint Lawrence Estuary (Canada).

No studies dealing with diameter of oocytes exists for *E. macha* and *M. edulis*. The mean diameter determined in this study for *T. dombeii* is smaller than that mentioned for other populations of the same species (Lozada, 1989). Interannual variability in mean size of oocytes may exist, as has been shown for *Macoma balthica* (L.) in Canada (Harvey et al., 1993) and *Mercenaria mercenaria* (L.) in South Carolina (USA) (Manzi et al., 1985).

In conclusion, there is variability in the timing of the gametogenic cycle of *E. macha*, *T. dombeii*, and *M. edulis* studied along the coast of southern Chile, i.e., the length of spawning and ripe stage periods varied. This is a key issue as far as management issues are concerned. Any management plan must take into account the geographic variability in the gametogenic cycles described here when closing seasons and minimum size of harvesting are determined. Due to the economic importance of many bivalves along the Chilean coast, there is an urgent need to evaluate the timing of gametogenic cycles of these species along their full geographic ranges.

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## Sclerochronology and Growth of the Bivalve Mollusks *Chione (Chionista) fluctifraga* and *C. (Chionista) cortezi* in the Northern Gulf of California, Mexico

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**Abstract.** Sclerochronology and analysis of oxygen isotopes reveal the age, growth rate, and growth patterns of *Chione (Chionista) cortezi* and *Chione (Chionista) fluctifraga*. *Chione (C.) cortezi* grows more quickly than *Chione (C.) fluctifraga*, but has a shorter life span (8 years versus 16 years). Microgrowth increments form with tidal periodicity, and their width is mostly influenced by temperature. Microincrement patterns reveal that maximum growth occurs from April to June and again in October. Growth is reduced during the hottest part of the summer and the coldest part of the winter. Growth breaks often occur in December/January and August. Timing of shell growth and environmental conditions were verified by high-resolution oxygen isotope measurements.

### INTRODUCTION

Bivalve mollusks of the genus *Chione* Megerle von Mühlfeld, 1811, inhabit many coastal areas around the world (Moore 1969:N686). Although they are often commercially exploited, little is known about their life span, overall growth patterns, and growth rates. The effect of temperature on the growth rate of *Chione* species is not well understood. Such information is important for the management of shellfish resources and mariculture.

Early attempts to determine the age and growth rate of mollusks used shell-weight or size-frequency analysis. The disadvantages of these methods have been reviewed by Berta (1976) namely: (1) the exact age of the youngest year class remains unknown; (2) year classes can be absent; and (3) size or weight ranges of specimens of different year classes can overlap due to differences in environmental conditions during their life. The major concentric rings on the external shell surfaces of mollusks have often been interpreted as annual growth patterns. However, they cannot always be distinguished unequivocally because rings may also be caused by non-seasonal disturbances. In addition, annual growth rings are crowded at the ventral margin in older specimens and make counting and interpretation difficult (see Zolotarev, 1980). Other researchers (e.g., Jones et al., 1978; MacDonald & Thomas, 1980) suggested that the most reliable method is to count the annual growth increments (first order increments or "1st-order layer" sensu Barker, 1964) preserved in radial cross-sections of the shells.

Since Wells's (1963) pioneering study, in which the microgrowth increments (higher order increments) of corals were used to infer the number of days in a Devonian year, many articles have dealt with the microgrowth increments of bivalve mollusks and other animals (for a review see Rhoads & Lutz, 1980 and references therein). This type of study has been termed "sclerochronology" (Buddemeier, 1975; Hudson et al., 1976).

Sclerochronology can be used to elucidate differences in growth rates and life histories of morphologically similar species. Sclerochronological methods can be applied to shells of fossil (Pannella 1976; Schöne, 1999) as well as living specimens. Organisms that produce accretionary hardparts serve as environmental recorders during their lives. Variation in growth rates and repeating growth structures have been interpreted to reflect endogenous rhythms, physiological periodicity, or environmental cycles. Varying widths of growth increments have also been attributed to random ecological fluctuations (e.g., Kennish & Olsson, 1975; Peterson, 1983). The stable isotope composition of growth layers is now being used in many paleobiological and paleoenvironmental studies (e.g., Tur-ekian et al., 1982; Williams et al., 1982; Roux et al., 1990; Kirby et al., 1998; Jones & Gould, 1999); and a few studies address the chemical content of the growth increments (e.g., Mutvei et al., 1994).

Here we present the results of stable isotope and sclerochronological investigations on the bivalve mollusks *Chione (Chionista) fluctifraga* (Sowerby, 1853) and *Chione (Chionista) cortezi* (Carpenter, 1864, ex Sloat MS) from the intertidal zone of the northern Gulf of California, Mexico. We describe inter- and intra-annual growth patterns and growth rates and interpret them in order to

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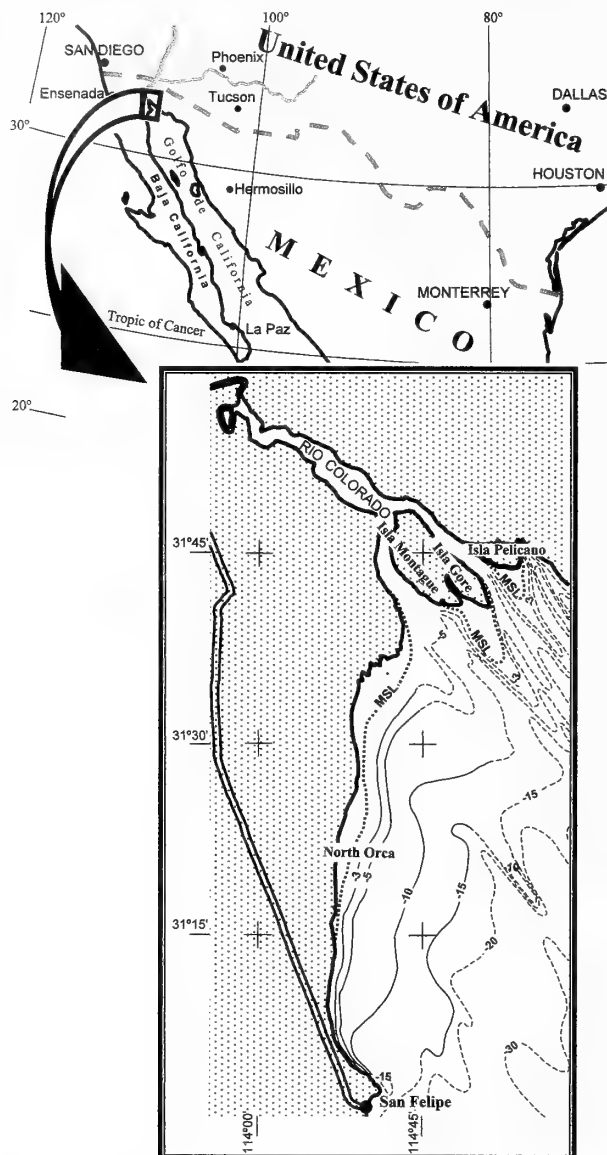


Figure 1. Sample localities in the Northern Gulf of California region. Samples were taken in mid-intertidal at low tide from North Orca ( $31^{\circ}20.087'N$ ,  $114^{\circ}52.957'W$ ) Isla Montague ( $31^{\circ}40.3'N$ ,  $114^{\circ}41.4'W$ ) and Isla Pelicano ( $31^{\circ}45.7'N$ ,  $114^{\circ}38.9'W$ ).

elucidate the life cycles and ages of the species. Intra-annual oxygen-isotope profiles provide further insight into temperature conditions during growth.

#### MATERIALS AND METHODS

More than 300 specimens of *C. (C.) cortezi* and *C. (C.) fluctifraga* were collected alive at low tide from the mid intertidal zone at three different localities in the northern Gulf of California, Mexico: North Orca, Isla Montague, and Isla Pelicano (Figure 1). Collecting was done in late

February 1997 and 1998, late May, early August, early November and early December of 1999. The tidal regime of the northern Gulf of California is semidiurnal with a mean tidal range of about 5 m. Average salinity of open Gulf water is  $38\text{‰} \pm 2\text{‰}$  in this area. Monthly average sea surface temperatures (SST) provided by satellite measurements (NOAA; <http://www.cdc.noaa.gov/>; WebWinds Java application, a software package to read the \*.netcdf files, was obtained from <http://webwinds.jpl.nasa.gov/>) vary between 15 and  $30^{\circ}\text{C}$ . Local temperatures may be 10 to  $15^{\circ}\text{C}$  higher or lower than those indicated by satellite measurements (personal observation).

Discrimination of the two species is often difficult. According to Villearreal-Chávez et al. (1999), *C. (C.) cortezi* is geographically restricted to the uppermost part of the Gulf of California, i.e.,  $31^{\circ}30'N$ , whereas *C. (C.) fluctifraga* is more widely distributed. Both species are recorded as present in the study area. According to Keen (1971), *C. (C.) cortezi* is larger and has a more trigonal form than *C. (C.) fluctifraga*. Smooth and polished concentric ribs characterize *C. (C.) fluctifraga*.

Fifty live specimens of *C. (C.) cortezi* were collected at North Orca (Figure 1) in early December 1999. Specimens were collected during four successive low tides in order to determine the time needed to produce the smallest shell growth increments. In addition, notching and staining experiments were conducted at the same location. Two-hundred specimens (120 *C. (C.) cortezi* and 80 *C. (C.) fluctifraga*) were held in buckets (also containing sediment from the collection site) in a tetracycline (1000 mg/L) or Alizarine Red solution (ambient seawater) in order to stain newly formed growth increments of the shells. Salinity ranged from 39 to  $43\text{‰}$  during the experiment. Forty specimens were sacrificed every 6 hours.

The flesh was removed from all shells immediately after collection to prevent further shell deposition. After coating with epoxy resin, valves were cut along the axis of maximum growth using a low speed (Buehler Isomet) saw, ground on glass plates (600, 800, and 1000 grit powder) and polished on laps (9, 6 and 0.3 microns) in order to enhance the contrast of the microstructures. Valves were ultrasonically rinsed in deionized water after each polishing step to remove grinding powder.

A caliper was used to measure the distances between major growth lines (i.e., annual increments) in radial cross-sections of 16 *Chione (C.) cortezi* and 18 *Chione (C.) fluctifraga* specimens (North Orca and Isla Montague material) to the nearest  $10\text{ }\mu\text{m}$  using a reflected light binocular microscope. Cumulative growth curves were calculated from the annual increment data, and each curve was fitted with a sigmoidal non-linear regression model referred to as "MMF model" in the software package CurveExpert Vs. 1.34 (available as shareware at <http://www.ebicom.net/~dhyams/cvxppt.htm>):

$$P(t) = (ab + ct^d)/(b + t^d),$$



where  $P(t)$  is the predicted increment width at time  $t$  and  $a$ ,  $b$ ,  $c$ , and  $d$  are fitted constants.

Thirty *Chione* (*C.*) *cortezi* specimens from North Orca and one *Chione* (*C.*) *cortezi* specimen from Isla Pelicano were etched in a NaOH-buffered EDTA solution (0.25 M, pH 7.9) for 1 to 2 hours, carefully rinsed in water and acetone, and allowed to air dry. Etching increased the contrast of the growth increments.

In addition, we prepared polished thin sections (thickness 30–50  $\mu\text{m}$ ) of five *Chione* (*C.*) *cortezi* specimens from North Orca and stained them with a 0.5% Rhodamine B solution for 3 minutes in the microwave at 50°C. This method stained the organic-rich microgrowth increments and made their recognition easier.

Microgrowth increment widths (i.e., higher order increments, from a few  $\mu\text{m}$  to 300  $\mu\text{m}$  thickness) of one *Chione* (*C.*) *cortezi* specimen (IP1-A1R) from Isla Pelicano were measured to the nearest 5  $\mu\text{m}$  in radial cross sections under a reflective light microscope using an eyepiece scale. We used linear regression (growth increment width versus growth increment number) to estimate how increment width changed through 1 year. The purpose of linear regression is to remove the age trend (Cook & Kairiukstis, 1990). The growth index was calculated by dividing the observed increment width by the predicted increment width. These procedures are known as detrending and indexing in dendrochronology (see Fritts, 1976; Cook & Kairiukstis, 1990). The growth index is a dimensionless measure of how growth deviates from the average trend. A value of 1 indicates no deviation; values greater than 1 indicate more rapid growth than expected; values less than 1 indicate slower growth than expected. Jones et al. (1989) have applied this technique to inter-annual growth variation of bivalve mollusks. Here, we apply the technique to intra-annual growth variation.

Specimen IP1-A1R was rinsed several times ultrasonically with deionized water prior to sampling for isotopic composition. The outer shell layer was sampled using a 300  $\mu\text{m}$  drill under a binocular microscope. The number of increments sampled varied from three to 40. Each of the 22 drill holes yielded 50 to 200  $\mu\text{g}$  of carbonate for isotopic analysis. A micromass automated carbonate extraction system was used to process the samples.  $\delta^{18}\text{O}$  is reproduced relative to PDB on a NBS-19 value of  $-1.92\text{‰}$ . Precision is better than 0.1 $\text{‰}$ .

## RESULTS

### Annual Growth Patterns and Growth Breaks

We observed two seasonally distinct interruptions in growth in both species. Samples collected in late February show a growth break (GB1, dark line, Figure 2d) near the ventral margin on the exterior shell surface as well as in radial cross sections. This pattern is more clearly developed in *C. (C.) cortezi* than in *C. (C.) fluctifraga*. No growth break occurs near the margin on specimens

collected in November or December. At greater magnification ( $\times 100$ ), the higher order increments preceding GB1 continuously decrease in width (Figure 2d). The nature of these higher order increments is described below in further detail.

A striking feature in both *Chione* species is a several millimeter-thick purple zone consisting of numerous, very narrow (approx. 1–5  $\mu\text{m}$ ) higher order increments (Figure 2c, e). In older specimens, there is a growth break (GB2, Figure 2b) visible within this purple zone that is expressed as a dark line on the outer shell surface. The thickness of this purple band varies with age and species. It is broader and less distinct in young specimens and it is more obvious in *C. (C.) fluctifraga* than in *C. (C.) cortezi*. The purple band is not seen after the last GB1 in May samples, but can be identified unequivocally at the ventral margin in specimens that were collected in early August. In these specimens, the purple band is thinner than in previous years recorded in the shell, suggesting that the GB2 was being deposited in early August.

The width of higher order increments increases continuously after GB1, reaches a maximum of 230  $\mu\text{m}$  about midway between GB1 and GB2, and then decreases slightly before the purple zone. This pattern is characteristic for specimens smaller than 3 cm. There is an additional small growth break in some larger specimens ( $> 4$  cm) usually somewhere between GB1 and the purple band. Growth rate decreases suddenly before this break and increases soon after.

The interval between the end of the purple band and GB1 is characterized by wider (up to 120  $\mu\text{m}$ ) microgrowth increments. Microgrowth increment width increases rapidly at the end of the purple zone. In specimens collected in early December, the higher order increments near the ventral margin are considerably smaller than those in specimens collected in November.

### Growth Rate

Increment widths between GB1's of both *Chione* species decrease from the umbo to the ventral margin, indicating that growth rate decreases with age. However, the growth curves are distinct for each species (Figure 3). Note that growth data from different localities from specimens living at different times are included in this diagram. Therefore, despite varying environmental influences, the overall growth patterns for each species remain essentially the same. Fitting the data with a sigmoidal growth function (MMF-model) returns very high correlation coefficients ( $r = 0.997$ ,  $P < 0.05$ ). Similar correlation coefficients have been reported from investigations of other species (Jones et al., 1989).

The two species differ in their maximum ages. Whereas the maximum observed age of *C. (C.) cortezi* specimens is 8 years, the oldest *C. (C.) fluctifraga* specimens are almost 16 years old. The oldest *C. (C.) cortezi* spec-

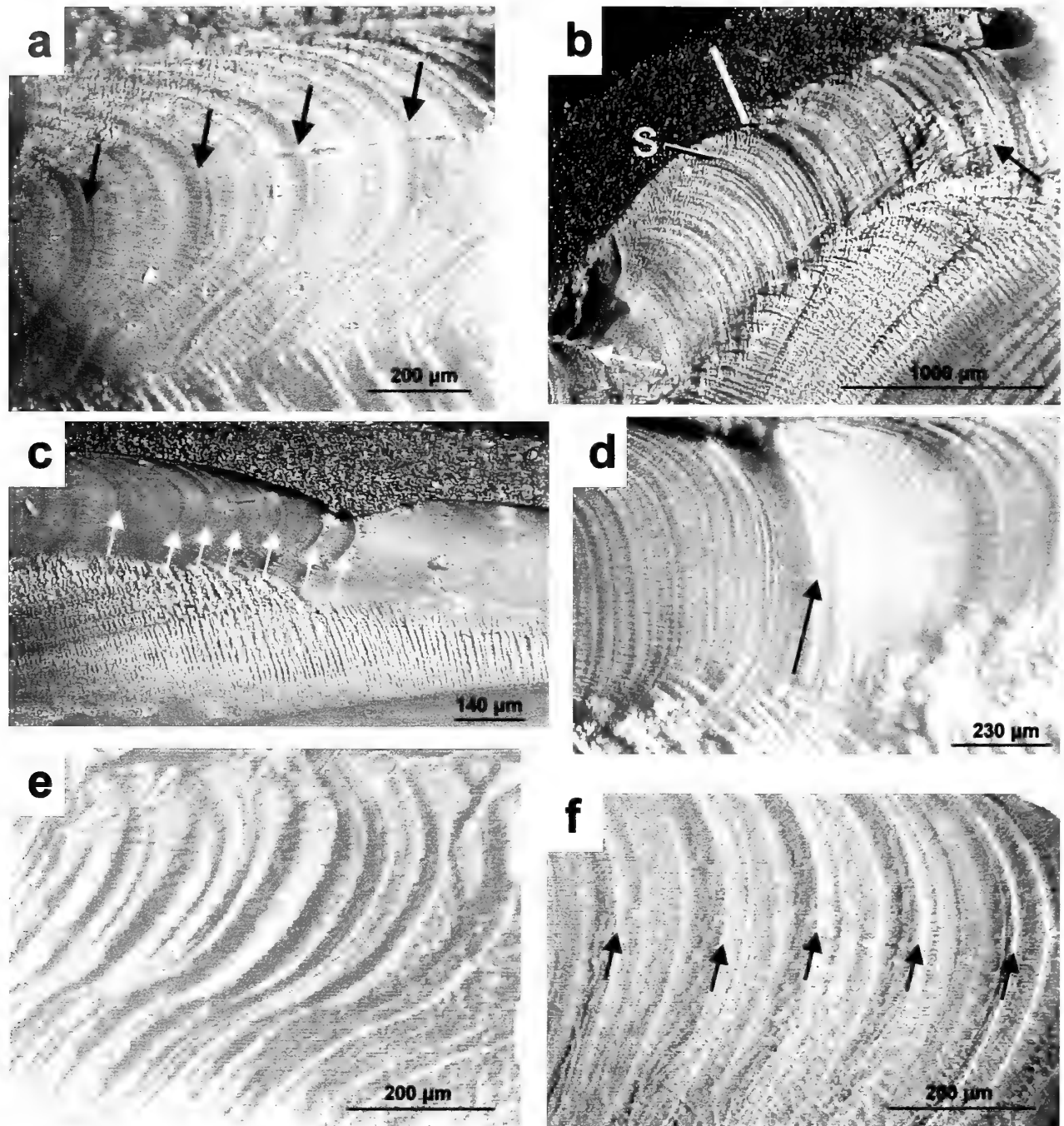


Figure 2. Microgrowth patterns of *Chione (Chionista) cortezi* (Figures 2a–d; sample no. IP1-A1R, Isla Pelicano) and *Chione (Chionista) fluctifraga* (Figures 2e, f; sample no. NO3-A6L, North Orca) as seen on etched surfaces under reflected light microscope (a–d) and SEM (e, f). Growth direction is always to the right. a. Lunar day increments produced during spring of the 3rd year. Each lunar day increment is bordered by thick ridges (arrows). Faint ridges are sometimes visible between two thicker ridges. b. Arrows mark an annual increment in a later ontogenetic stage (6th year). The white line indicates the summer break (GB2). The spawning break is indicated by "s." c. Slowdown of growth in the summer in an early ontogenetic stage (3rd year). Fortnightly cycles are indicated by arrows. d. Winter break (GB1, arrow) in the 3rd year. Note the narrow increments preceding the break and their increasing width after the break to the right. e. Growth slowdown during hot summer. Lunar day increments are about  $\frac{1}{4}$  the width of those earlier in the spring of the same year. (shown in Figure 2f). The etch-resistant increments are broader than during the spring and fall. f. Lunar day increments (see Figure 2a for description).

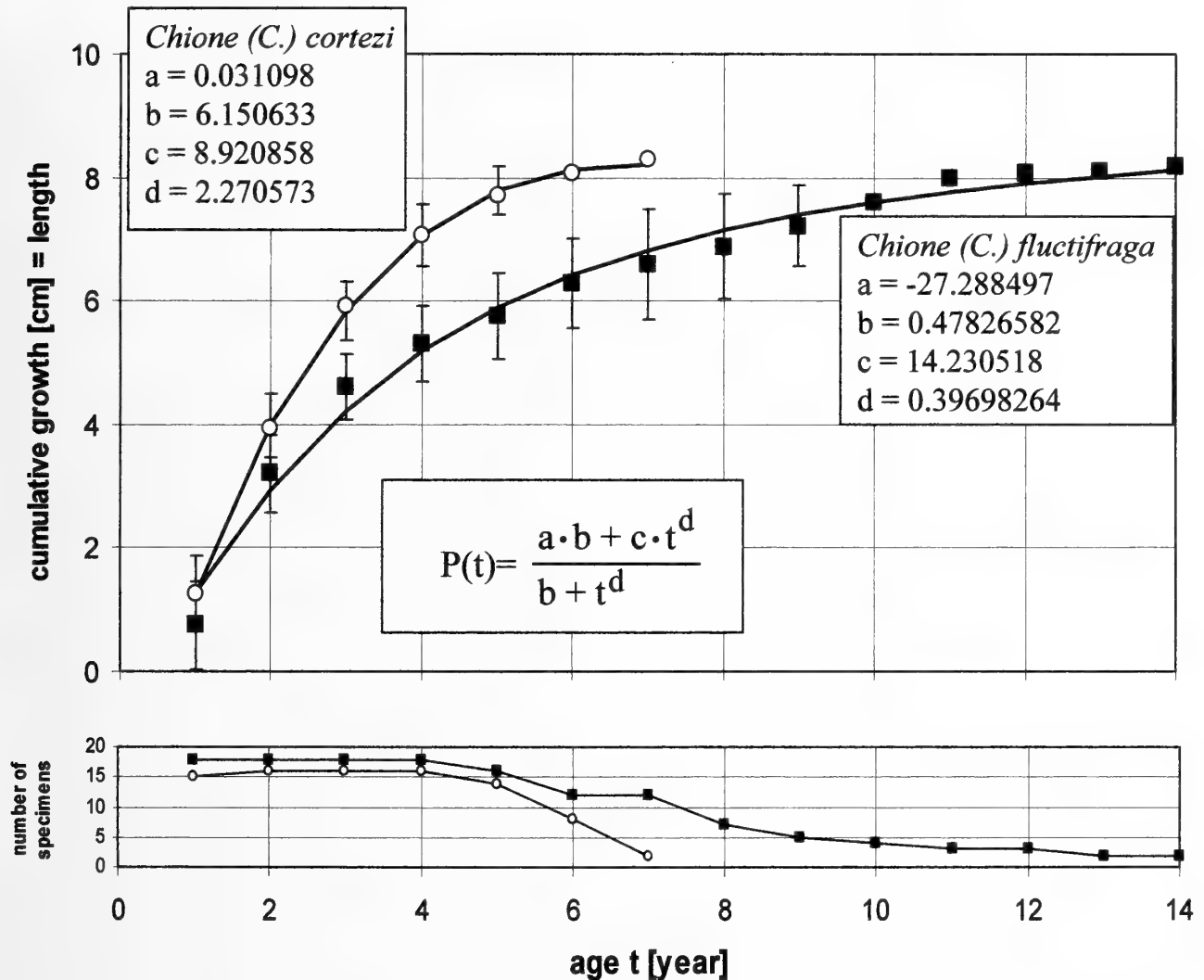


Figure 3. Growth curves of *Chione (Chionista) cortezi* and *Chione (Chionista) fluctifraga*. Maximum observed ages in *Chione (Chionista) fluctifraga* are lower than in *Chione (Chionista) cortezi*. *Chione (Chionista) cortezi* grows faster than *Chione (Chionista) fluctifraga*.

imens, however, are generally  $\frac{1}{3}$  larger than the oldest *C. (C.) fluctifraga* specimens. The ventral margins of the oldest *C. (C.) fluctifraga* specimens are bent to the inside: the growth direction in this species changes from an anterior-posterior direction to growth directed toward the opposite margin along the commissure. With increasing age, the shell margin of *C. (C.) fluctifraga* becomes obtuse, and the convexity of its shell increases sharply as has been demonstrated by Zolotarev (1980) for other species. As a result, the length/height relationship of old specimens is higher than that of young specimens.

#### Higher Order Growth Increments

Cross-dating (matching increments in different specimens, see Fritts, 1976) of the most recently produced increments on the ventral margin of 14 *Chione (C.) cortezi*

and eight *Chione (C.) fluctifraga* specimens collected at North Orca in early December 1999 indicates that every high-low tidal cycle results in a couplet of one narrow etch-resistant and one broader, deeply etched increment. A high-low tidal cycle comprises approximately 12.4 hours. In specimens collected during the morning low tide, the etch-resistant increment at the commissure is considerably less distinct than in specimens collected during afternoon low tide. The time interval between two thick etch-resistant growth increments is approximately 24.8 hours. In the paleontological and biological literature, the term lunar day is often used to describe the time interval between these microgrowth increments (e.g., Evans, 1972; Pannella, 1976). A lunar day is the amount of time required for one rotation of the Earth on its axis, with respect to the Moon.

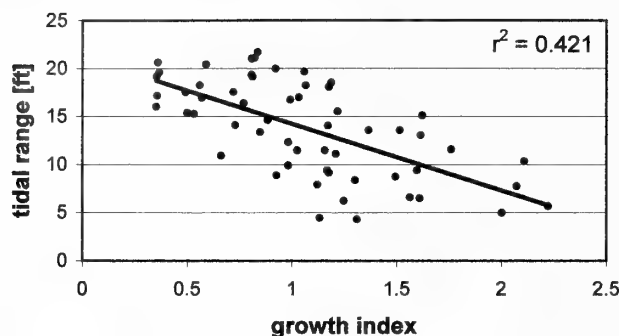


Figure 4. Relationship between tidal range and growth index. Maximum growth rate (here shown for *Chione (Chionista) cortezi*, shell IP1-A1R) corresponds to low tidal range, i.e., neap tides. Note that the influence of temperature on growth has not been extracted from the growth index.

Counting lunar days from the ventral margin of three *Chione (C.) cortezi* specimens (one from Isla Pelicano and two from Isla Montague) back toward the umbo reveals a growth pattern that coincides with lunar tidal cycles similar to what was noted by Evans (1972) for other species. Tidal range and shell growth are negatively correlated (Figure 4). Maximum growth rate occurs during neap tides when tidal range is low. Two relatively narrow lunar day increments form in a fortnightly cycle ("3rd-order layers" *sensu* Barker, 1964). They are most probably formed during spring tides. The small increments are accompanied by growth depressions on the external shell surface (Berry & Barker, 1968).

Twenty stained (tetracycline 1000 mg/L, Alizarine Red; bucket experiments) specimens of both *Chione* species show a yellow-orange (tetracycline under UV-light) or reddish band, whose widths correspond to the increments formed during exposure to the stains. These experiments confirm the results found by field sampling on consecutive tides (described above). Only specimens of age-class one and two, however, showed noticeable shell growth in December 1999 when these experiments were conducted.

All these findings enabled us to date the major events in the shell with precision to the nearest fortnightly cycle. However, the total number of lunar days within an annual increment was always less than the expected number (353.25) of lunar day increments in a solar year. The total number of lunar day increments in three specimens of *C. (C.) cortezi* was 253, 291, and 307.

### Stable Isotope Variation

The oxygen isotope composition of a shell is a function of the ambient temperature and  $\delta^{18}\text{O}$  of the water (which in turn is determined by evaporation rate and the amount of freshwater input) in which the individual is living. Shells were collected at times when the Colorado River did not flow into the Gulf of California. Thus changes in

shell  $\delta^{18}\text{O}$  are a function of changes in temperature and evaporation but not fluvial influx (see Dodd & Stanton, 1990, for an extensive discussion).  $\delta^{18}\text{O}$  values vary with the inverse of temperature: high  $\delta^{18}\text{O}$  values indicate low temperatures and low  $\delta^{18}\text{O}$  values indicate high temperature. For aragonitic mollusks a temperature increase of 4.7°C results in an isotopic shift of 1‰ (Grossman & Ku, 1986).

$\delta^{18}\text{O}$  values in the third year of growth of *C. (C.) cortezi* specimen IP1-A1R range from 0.91 to -2.47 (Figure 5c), corresponding to a temperature of 15.5 to 30.5°C. Values are highest in shell material deposited during winter and are lowest in the purple band deposited between mid July and mid September.

## DISCUSSION

### Annual Growth and Growth Breaks

Periodic, distinct growth patterns ("biochecks" of Hall et al., 1974), i.e., seasonal growth halts, growth retardation, or structural change of material form the basis for a chronology, based on shell growth. Biochecks segment the growth increment pattern into time intervals of approximately equal duration and can be used for many purposes, including determining the age of an individual bivalve mollusk.

Because seasonal events do not recur at exactly the same time each year, the number of increments per annual increment may differ. Therefore, Hall et al. (1974) introduced the term "median date of the deposition of biochecks." Biochecks are usually related to temperature extremes (low or high; e.g., Davenport, 1938; Pannella & MacClintock, 1968; Kennish & Olsson, 1975; Clark, 1975; Jones, 1983) and to spawning events (Jones, 1980; Sato, 1995; and references therein). Caution should be exercised when using reproduction biochecks in different specimens for dating. The dates of reproduction breaks in *Chione (C.) fluctifraga* vary considerably between individuals (Martínez-Córdova, 1988). This has also been shown for other bivalve mollusks (e.g., Coe, 1948; Coe & Fitch, 1950; Sato, 1995).

Depending on the seasonal temperature cycles, one or two temperature-mediated biochecks can be present: a summer break and/or a winter break (e.g., Koike, 1980; Clark, 1979; Sato, 1995; Jones & Quitmyer, 1996). The specimens studied here show both a winter and a summer biocheck (compare Koike, 1980 and references therein). The winter break represents a cessation of growth. The summer break represents a slowdown and/or a cessation.

The growth slowdown in summer is macroscopically expressed as a purple band (summer band). In some shells a growth halt is present within the purple band (summer break, GB2). The shutdown of growth in the cold season is called a winter break (GB1). Additional support for this interpretation comes from counting the lunar day increments in specimens collected during different seasons to

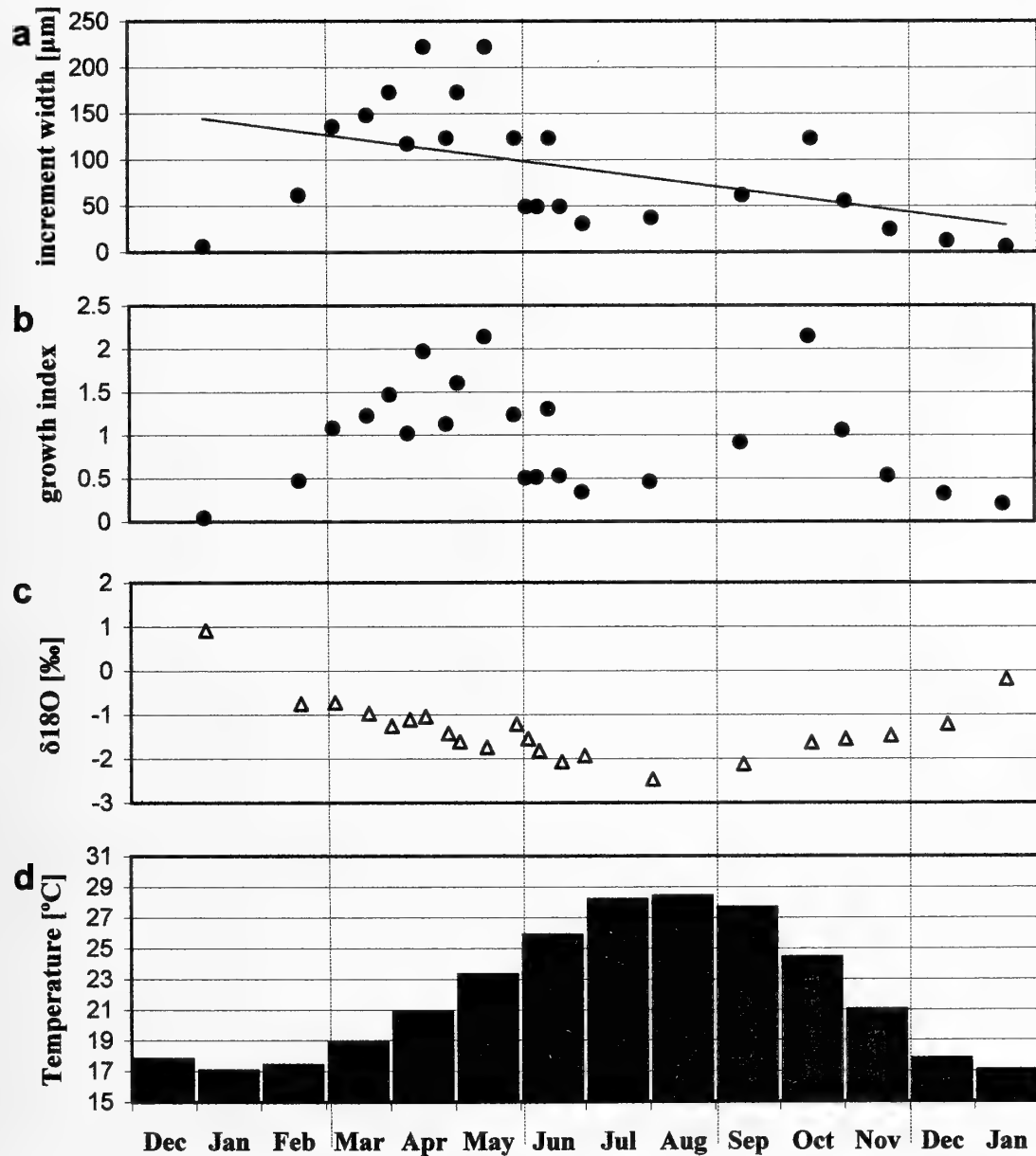


Figure 5. Comparison of growth rate and oxygen isotope composition of *Chione* (*Chionista*) *cortezi* (IP1-A1R) and sea surface temperatures. a. Microgrowth increment width (lunar day increment width). Only those data are depicted for which oxygen isotope composition has been determined. A linear fit has been applied to the raw data in order to extract the inherent age trend. b. Age-detrended growth data. The residuals were calculated from the data in Figure 5a by dividing the measured by the predicted (linear fit) data. c. Oxygen isotope data of selected increments. d. Monthly sea surface temperatures (SST) based on satellite observations of the sampled region during growth of *Chione* (*Chionista*) *cortezi*.

date the biochecks. In the Gulf of California, unfavorable temperature extremes for *Chione*'s growth are reached during both summer and winter. Because these temperatures can be reached more than once during each season, there may sometimes be several growth halts within a winter or summer band.

Annual reproduction breaks occur in specimens 3 years

old or older. These show the characteristic microgrowth pattern described by Kennish & Olsson (1975) and Sato (1995) for reproduction breaks, namely abruptly smaller increment widths that preceded the break followed by broad increments afterward (Figure 2b). The interpretation of these late spring or early summer growth biochecks as reproduction events is reinforced by counting

lunar day increments, e.g., most specimens of both species start their first year of growth in late spring or early summer, indicating that spawning occurred shortly before. Of all collected specimens in early November and December only 10 are clearly younger than 2 months, indicating that spawning in late summer is rare. Histologic studies on gonad development of *Chione* (*C.*) *fluctifraga* specimens by Martínez-Córdova (1988) also indicate spawning in spring.

### Growth Rate

Growth curves for the two *Chione* species, based on annual growth increment measurements, are similar to those published for other bivalve mollusk species (e.g., Sato, 1994, 1995; Hall et al., 1974; Thompson et al., 1980; Jones et al., 1978). Rapid growth occurs during early ontogenetic stages, and growth rates decrease as the individuals mature (Figure 3). This pattern is best described with a sigmoidal growth equation. Other investigators have found an exponential relationship between age and growth rate in various bivalve mollusk species (von Bertalanffy, 1934; Hall et al., 1974; Richardson et al., 1980; Jones et al., 1989; Mutvei et al., 1994).

### Higher Order Growth Increments

Staining experiments and specimens collected on consecutive tidal cycles reveal unequivocally that a couplet of two etch-resistant increments and two deeply etched increments (Figure 2a, f) are produced each lunar day. The etch-resistant increments are more prominent if high temperatures prevail at low tide (Figure 2e). The width of the deeply etched increments increases with temperature, but is reduced above and below specific temperature extremes, both cold and warm (see below).

Crabtree et al. (1980) found that growth increments in *C. (C.) fluctifraga* are a poor indicator of time. Crabtree et al. (1980) conducted notching experiments on *C. (C.) fluctifraga* specimens. They concluded that "the line counts did not agree well with the number of days in the growth period" between notching and recovery. Furthermore, they found that there was no "consistency in growth line counts" both between different counters and between specimens of different age classes. However, a careful re-examination of the young specimen depicted in figure 8 of their paper shows 45 to 47 couplets consisting of two dark and two light increments. This should correspond to 45 to 47 lunar days consisting of 90 to 94 dark growth lines, although the six persons in their experiment counted only 56 to 74 lines (i.e., 23 to 37 lunar days). Our recounts match the expected number of increments (~48 lunar days) very well.

Furthermore, recalculation of the tidal increment cycles (with wxtide25, Windows program available at [http://www.geocities.com/Silicon\\_Valley/Horizon/1195/wxtide32.html](http://www.geocities.com/Silicon_Valley/Horizon/1195/wxtide32.html)) indicates that the slight depressions (= deeply etched in-

crements) between the growth ridges (etch-resistant increments) begin to form at or just after neap tides (Crabtree et al., 1980: figure 8). Berry & Barker (1968) were the first to suggest a fortnightly periodicity in the formation of external growth ridges in *Chione*.

The time interval that a lunar day increment—whether solar (light/dark cycle) or tidal (Barker's, 1964, "4th-order layer")—represents is controversial. There is little evidence when and in which time period the etch-resistant and deeply etched parts of the increments are produced (but see Richardson et al. 1981). Our experiments were not able to clarify this problem.

### Stable Isotope Variation

Values of  $\delta^{18}\text{O}$  vary inversely with ambient sea surface temperatures (Figure 6c, d). The 3.38‰ annual range in  $\delta^{18}\text{O}$  values corresponds to a 15.9°C temperature range ( $3.38\text{‰} \times 4.7^\circ\text{C}/\text{‰}$ , see Grossman & Ku, 1986). This range is greater than the 11.4°C maximum difference in mean monthly SSTs observed by satellite. However, the SST data are monthly averages, and the difference between maximum and minimum daily temperatures will exceed the difference in monthly averages. The isotopically determined temperature range represents the range of temperature during which shell growth occurs, not the total range during the year, because growth ceases during seasonal temperature extremes.

### Temperature Control of Growth Rate

As discussed above, the growth rate of *Chione* (*C.*) *cortezi* varies seasonally (see Figures 5a, b). Growth rate is high from March to June, decreases from July to September, increases again in September and October, slows in November and December, and halts during late December. Growth starts again late in February. This pattern suggests that both low and high temperatures inhibit growth in this species. Maximum growth rates occur when monthly average temperatures are between 21 and 24°C (Figure 6). Ninety-five percent of the annual increment width is formed between 16.7 and 29.3°C (monthly average SST, satellite data, Figure 5d). Isotopically derived estimates of temperature confirm this range. Winter growth breaks (GB1) occur when temperatures drop below this range and summer breaks (GB2) occur when temperatures exceed this range.

### SUMMARY AND CONCLUSIONS

Specimens of *Chione* (*C.*) *fluctifraga* and *Chione* (*C.*) *cortezi* show both a winter and summer biocheck. Both biochecks are useful for ontogenetic age determination. The maximum observed age for *Chione* (*C.*) *fluctifraga* is higher (15 years) than that of *Chione* (*C.*) *cortezi* (8 years) even though *Chione* (*C.*) *cortezi* grows to a larger size. *Chione* (*C.*) *fluctifraga* grows much more slowly than *Chione* (*C.*) *cortezi*.



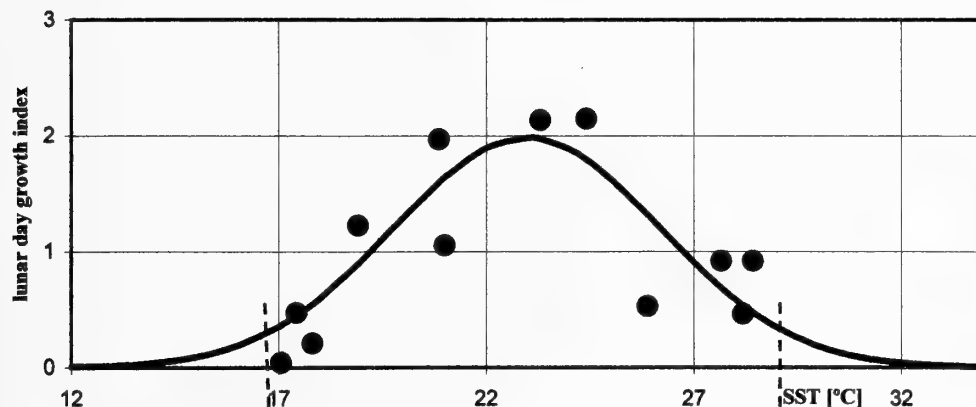


Figure 6. Growth indices (lunar days) and SST fitted with a Gaussian function. The dashed lines indicate the upper and lower growth temperature thresholds in *Chione (C.) cortezi* and *Chione (C.) fluctifraga*.

Growth breaks accompanied by dark lines on the exterior shell surface are commonly observed within the winter and summer bands. Furthermore, some specimens exhibit a spawning break in late spring.

Microgrowth increments form with tidal rhythms and are useful for dating special events (summer, winter, tidal cycles, storms, spawning, etc.).

Maximum growth rates occur during April to June and again during October. Growth occurs between February and December and is suppressed by temperature extremes both during the cold season and the hot summer period (mid July to mid September).

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## The Influence of Hunger and Olfactory Cues on the Feeding Behavior of the Waved Whelk, *Buccinum undatum*, on the Blue Mussel, *Mytilus edulis*

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**Abstract.** A marine prosobranch gastropod, the waved whelk *Buccinum undatum*, is a ubiquitous predator in the North Atlantic. Previous studies have shown the whelk's diet to consist primarily of bivalve mollusks that the whelk can open using the lip of its shell as a wedge. This experiment investigated the circumstances under which whelks will attempt to feed upon the blue mussel *Mytilus edulis*. Whelks did not attempt to feed upon intact mussels after a period of starvation of 2 weeks, but were significantly more likely to attempt to feed after 6 weeks starvation. However, because of the mussel's ability to close so tightly, this was usually unsuccessful. Whelks starved less than 6 weeks attempted to feed only on mussels that had sustained tissue damage. Whelks were also attracted to water from injured mussels. This suggests that, despite the relatively high abundance of blue mussels, whelks feed on these mussels only opportunistically. This supports the argument that *B. undatum* is primarily a scavenger and has only limited success as a predator upon healthy bivalves.

### INTRODUCTION

The marine prosobranch gastropod, the waved whelk *Buccinum undatum* (Linnaeus, 1758), is abundant in the North Atlantic. It tolerates a wide range of salinity and is found in depths as shallow as the mean low water mark and as deep as 1000 m (Brock, 1936; Fretter & Graham, 1962). It is considered to be a carnivore with a tendency to scavenge (Blegvad, 1915). Prior studies of stomach analysis have shown the whelk's diet to be dominated by bivalve mollusks (Nielsen, 1975). After an extensive study of *Buccinum* predation on many species of bivalves, Nielsen (1975) concluded that only rarely were the whelks able to overcome healthy bivalves of most species—including *Mytilus edulis*, the blue mussel. The whelk's method of attack is to crawl upon the shell and orient itself so that the anterior margin of the foot is in contact with the ventral edge of one of the shells of the bivalve. If the bivalve is open after this procedure, or if it reopens again after the whelk has settled, the whelk suddenly contracts and forces the lip of its shell in between the valves of the bivalve, preventing its closure. If the resulting opening is large enough, the whelk will then insert its proboscis and begin tearing flesh with its radular apparatus (Nielsen, 1975). Nielsen observed that *Buccinum* was rarely able to overcome healthy bivalves of most species. *Mytilus edulis* (Linnaeus, 1758) and *Modiolus modiolus* (Linnaeus, 1758) were able to close tightly enough that the whelks usually gave up any attempts to

open and crawled away. At times during an attempt to open, the mussels would close so tightly upon the lip of the whelk's shell as to cause it to break. One can find many whelks in a population with such scars, indicating that the process of preying upon healthy bivalves is difficult, dangerous, and potentially energetically expensive.

This experiment examined the circumstances under which whelks will attempt to feed on blue mussels. I examined their motivation to feed after 2 and 6 weeks of starvation to test the hypothesis that whelks should only attempt to feed upon healthy blue mussels after a sufficient period of starvation. I also examined the differences between whelk feeding attempts on mussels that had sustained tissue damage and those that had not sustained tissue damage. Finally, I examined the olfactory cues necessary to stimulate feeding behavior.

### MATERIALS AND METHODS

Whelks were collected from the subtidal Gulf of Maine waters in the vicinity of the Isles of Shoals, New Hampshire; along the coast of New Castle, New Hampshire; and in Eastport, Maine. They were kept in filtered seawater at 10°C. Each whelk was used once in experiments and then released. The prey species *Mytilus edulis* was collected from intertidal and subtidal locations along the New Hampshire and Maine coastlines. Prey specimens were also kept in filtered seawater at 10°C. Mussels used were between 1.2 cm and 6.3 cm in length.

The whelks were fed fresh, pre-opened *M. edulis* at a designated time prior to each experiment. This was followed by a period of food deprivation to establish a uniform level of hunger in all whelks used in that particular trial.

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Table 1  
Feeding cues for *Buccinum undatum*.

		# Attempts to feed	# No attempts to feed
Effect of hunger	2 weeks starvation	0	18
	6 weeks starvation	5	3
Feeding stimuli	MgCl <sub>2</sub> <i>Mytilus</i> treatment	0	5
	Healthy <i>Mytilus</i>	0	12
	Nicked tissue <i>Mytilus</i>	4	4
		Arousal	No arousal
Effect of prey-specific olfactory stimuli	<i>Mytilus</i> extract	4	0
	Seaweed extract	0	8

Whelks' responses were scored in four categories: no attempt to feed, unsuccessful attempt, successful attempt, and "arousal," which was indicated by a major postural change or locomotion in response to a stimulus.

#### Experiment A—Effect of Hunger

Whelks ( $n = 26$ ) were each isolated into 9.45 L aquariums filled with filtered seawater. Each aquarium had an air stone. Whelks were fed once with a pre-opened, medium-sized (relative to the whelk) *M. edulis*, which they consumed. They were then kept without food for 2 or 6 weeks before the feeding trial. The effect of hunger was then examined by presenting individual whelks with a live, healthy *M. edulis*, and observing them for 2–3 hours for any attempts to feed.

#### Experiment B—Effect of Prey Condition

In this experiment, whelks that had not been fed for 3 weeks were either given a live, healthy, normal *Mytilus* or one that had been weakened using a 3.5% solution of MgCl<sub>2</sub> in seawater. The mussels were placed in the treatment solution for 15 minutes. This treatment affected the nervous system of the mussels, so that when touched or handled they remained in an open relaxed position and did not close their valves. This condition lasted 30 minutes, which was sufficient for the experiment. Outcome was scored in the same fashion as in experiment A.

I examined responses of whelks that had not been fed for 3 weeks that were given healthy mussels, and whelks given mussels with slight tissue damage. The mussels were nicked slightly on their mantle with a razor, but their adductor muscles were left intact. Outcome was again scored in the same fashion as in experiment A.

#### Experiment C—Effect of Chemical Cues

Chemoreception is the primary means by which whelks locate food (Brock, 1936). To identify the source of olfactory cues that induce feeding, I used mussel "scent." To produce mussel scent, a mussel was opened with a

razor and placed in 25 mL of distilled water for approximately 20 minutes. Seaweed "scent" was used as a control and was created by crushing a few grams of *Laminaria* sp. in 25 mL of distilled water. Whelks were not fed for 3 or 5 weeks before testing. Whelks were placed in the center of their respective tanks. Next, 1 mL of either mussel "scent" or seaweed (*Laminaria* sp.) extract was added to the water in the vicinity of the whelk. Since no actual prey items were present during this experiment, whelk responses were scored as either "arousal" or no response. Aroused whelks showed an immediate postural change often followed by locomotion directed into the current and toward the scent plume. Unresponsive whelks remain stationary and relaxed with their shell resting upon the substrate.

## RESULTS

**Experiment A:** Whelks ( $n = 18$ ) deprived of food for only 2 weeks never attempted to feed, whereas those ( $n = 8$ ) starved for 6 weeks were significantly more likely to attempt to feed on healthy mussels (Fisher's exact test  $P = 0.001$ , Table 1).

**Experiment B:** Whelks made no attempt to feed on mussels subjected to a MgCl<sub>2</sub> treatment ( $n = 5$ ) or untreated mussels ( $n = 12$ ). However, when offered mussels that had sustained mantle tissue damage (with the adductor muscles still intact), significantly more whelks attempted to feed ( $n = 8$ ) (Chi-square goodness-of-fit test  $P = 0.002$ , Table 1).

**Experiment C:** Whelks showed a significant response to prey-specific odors. All showed a typical arousal response to mussel scent ( $n = 4$ ), and none did to seaweed scent ( $n = 8$ ) (Fisher's exact test  $P = 0.002$ , Table 1).

## DISCUSSION

Behavior is contingent upon both the external conditions and the internal state of the animal. In foraging, the decisions made regarding when and how to feed often re-

flect the hunger of the animal—the internal variable, and the food availability—the external variable. In this case, whelks preferred damaged prey, but when faced with the risk of starvation as a result of restricted food availability, there was a shift in the foraging decision. That is, the hungry whelks were more likely to attempt to open a healthy mussel.

Nielsen (1975) showed that healthy mussels have an excellent defense against whelk predation in their ability to close their valves tightly for long periods of time. Opening an intact mussel is energetically expensive and presents a risk of injury. Thus feeding upon a healthy bivalve does not constitute as large a net energy gain as feeding upon a damaged bivalve. This suggests two things: first, injured prey would be preferred to intact prey; and second, the whelk would attempt to feed on a healthy bivalve only as the risk of starvation increases. Both of these predictions are supported by the data.

The inverse relationship between hunger and selectivity is widespread. Feeding preferences of another predatory gastropod, the dogwhelk *Nucella lapillus* (Linnaeus, 1758), have also been shown to be influenced by starvation. Dogwhelks restricted from feeding were more likely to feed upon a patch of barnacles (Vadas et al., 1994). Likewise, a study of the predatory snail *Acanthina spirata* (de Blaineville, 1832) showed the species to be less selective between two different barnacle species when starved (Perry, 1987).

The behavior of *Buccinum undatum* can be fitted to a risk-sensitive model of behavior. Here, the term risk refers to probabilistic variation in prey (Stephens & Krebs, 1986). Caraco et al. (1980) demonstrated that an animal's energy budget can predict whether it is risk-averse or risk-prone. That is, an animal on a negative energy budget (a hungry whelk, for example) will prefer variable food rewards. In this case, a variable food reward is a healthy mussel. An attempt to open it will result in either a large gain in energy or none at all. On the other hand, an animal on a positive energy budget will prefer less variable food rewards—in this case, a damaged mussel for which there is no chance of failure if the whelk attempts to feed. Stated simply, hungrier animals are more impulsive and thus less selective with regards to foraging opportunities (Synderman, 1983). It is possible that starvation, in addition to reducing the selectivity of whelks, has a negative effect on the successes of attacks upon bivalves. If this is the case, there may come a point at which prey selectivity increases again, as any further attempts to open healthy bivalves would prove unsuccessful. An investigation on the effects of starvation and diet of another species of whelk, *Bullia digitalis*, demonstrated that starvation had a detrimental effect upon the feeding process in some cases (Stenton-Dozey et al., 1995).

The cues a whelk uses to make a decision to feed are important. Odor cues from damaged mussel tissue are

sufficient to trigger feeding behavior, whereas odor cues from undamaged tissue are not. Although the mussels treated with  $MgCl_2$  were defenseless, no whelks attempted to feed, even after investigating the mussel and encountering no defensive response. However, damaged mussels that could still close tightly were attractive prey. Chemosensory cues from the damaged tissue are important to stimulate feeding behavior, whereas visual or tactile cues seem to be of little importance. When mussel scent alone was presented, all whelks showed the postural change associated with the stimulus of the mussel extract that suggested interest in feeding. None showed any interest or response to an equivalent addition of seaweed extract to the water, demonstrating that whelks distinguish prey odors from non-prey odors in the water, and react accordingly.

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## Larval Development, Precompetent Period, and a Natural Spawning Event of the Pectinacean Bivalve *Spondylus tenebrosus* (Reeve, 1856)

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**Abstract.** The artificial induction of spawning, and the development of larval *Spondylus tenebrosus*, a spondylid pectinacean bivalve, is described. A combination of warming and injections of serotonin into the adductor muscle of these animals produced spawning within 1.5 hours. Larvae were cultured at temperatures ranging from 22–24°C. Pediveligers were first observed within 12 days of fertilization, and settlement was first observed 21 days after fertilization.

Pediveligers that were not allowed to settle were healthy 60 days after fertilization, when culture ended, indicating that larval *S. tenebrosus* can delay settlement, and remain planktonic for at least 2 months. In addition, a spawning event of natural populations on the southern shore of Oahu, Hawaii was indirectly observed. This spawning event occurred during a period of maximum annual temperature, suggesting that warming may be a natural spawning cue for *Spondylus tenebrosus*.

### INTRODUCTION

*Spondylus tenebrosus* (Reeve, 1856), a spondylid pectinacean bivalve, is a commonly recruiting bivalve in Hawaiian coastal waters (Bailey-Brock, 1989). *S. tenebrosus* occurs in Australia, and the Gilbert, Marshall, and Hawaiian Islands (Kay, 1979). In Hawaii, it mainly occupies vertical surfaces of natural and artificial reefs, and the upper surfaces of holes and small caves (Thorsson, 1987). It occurs from the shallow subtidal zone to at least a depth of 40 meters.

Pectinacean larval development is similar to that observed in many bivalves (Cragg & Crisp, 1991). Gametes are spawned into the water column, and fertilization is external. Cleavage stages develop into mobile ciliated trochophores. The first shelled stage is prodissoconch I, and it is during this stage that the larvae first feed on phytoplankton. Prodissoconch II (umbone) larvae are characterized by the initiation of umbone development and the appearance of commarginal growth lines. The pediveliger stage, identified by the presence of a foot, is competent to settle. Pediveligers may leave the water column and crawl on the bottom using this foot prior to settlement.

The larval developmental period of pectinaceans, as observed in previous lab studies, varies with temperature and salinity, and among species. Delayed growth and development of pectinacean larvae have been observed at decreased temperatures (Beaumont & Budd, 1982). Pectinacean precompetent periods range from 14 days, for

the tropical pectinacean *Amusium pleuronectes* (Belda & Del Norte, 1988), to more than 42 days for the temperate pectinacean *Chlamys hastata* (Strathmann, 1987).

Although larvae of *Spondylus tenebrosus* are common in the Hawaiian coastal plankton throughout the year, the larval development of this species has not been previously described. The period between the initial release of gametes and the development of larvae that are capable of settlement is referred to here as the precompetent period. The goal of the present study was to determine the minimum precompetent period of *S. tenebrosus*, and thus determine the minimum period that larvae are planktonic and subject to dispersion by circulation. This information was needed to supplement a field study of benthic invertebrate recruitment pattern forced by the interaction of circulation, larval planktonic period, and adult distribution (work in progress).

### MATERIALS AND METHODS

#### Broodstock

Adult *S. tenebrosus* were collected twice from an artificial reef off Waikiki Beach (southern shore of Oahu, Hawaii) at a depth of 35 meters using SCUBA. Six individuals were collected on 10 August 1996 and were dissected immediately to check for gonad condition. Five individuals had ripe gonads (four females and one male). This initial collection was conducted to determine if animals were available and, if so, to check their gonad condition. A second collection was conducted after preparations were made to culture adults and larvae. Sixteen adults were collected on 21 September 1996 for broodstock. These animals were transported to the lab in a 128 L cooler, half filled with surface water from the collection

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site ( $T = 26.7^{\circ}\text{C}$ ). The animals were cleaned of fouling algae and invertebrates using a plastic scrubbing pad and a putty knife. They were then placed in a 70 L aquarium filled with seawater at  $\sim 26^{\circ}\text{C}$ . The aquarium temperature was kept constant until spawning induction was attempted 2 hours later.

### Spawning

The broodstock was placed in a 3 L glass bowl filled with  $0.2\ \mu\text{m}$  filtered seawater (FSW) for spawning (salinity  $\approx 34$ ). Spawning induction was first attempted by injection of  $0.5\ \text{mL}$  of a  $2\ \text{mM}$  serotonin solution (Strathmann, 1987; Monsalvo-Spencer et al., 1997; Rhee & Davis, 1997). Serotonin was injected through  $\sim 2\ \text{mm}$  holes that were drilled through the anterior valves so that adductor muscles could be injected; one hole was drilled per animal. Injections were not possible without drilling because the animals rapidly closed their valves upon sensing any motion. Several animals opened and closed their valves quickly within 2 minutes of injection, but no gametes were seen exiting the mantles. No spawning was observed for 2 hours. The animals were then warmed from  $26.2$ – $35.0^{\circ}\text{C}$  over a 1-hour period (salinity  $\approx 34$ ) as a second attempt to induce spawning; spawning did not occur. Five individuals were then dissected to obtain gametes, but all gonads were empty. It was therefore likely that the *S. tenebrosus* population on the artificial reef off Waikiki had spawned since the first collection of 10 August.

Two different populations of *S. tenebrosus* in Mamala Bay were then sampled on 23 September to check for gonad condition. Five animals were taken at a depth of 3 meters from a sunken barge off the west end of Waikiki Beach, and five animals were collected at a depth of 30 meters from a natural reef off Ewa Beach. These animals were dissected upon return to the lab, and none had ripe gonads.

The 11 individuals that remained from the 21 September collection were maintained in a 60 L aquarium filled with natural seawater at  $\sim 26^{\circ}\text{C}$ . The animals were fed a mixed diet of phytoplankton in an attempt to return them to spawning condition. The algal diet was composed of dense suspensions (algal concentrations were not quantified) of *Isochrysis galbana* (Tahitian strain) and *Skeletonema costatum* (Greville) at a temperature of  $\sim 26^{\circ}\text{C}$ . On 21 November (60 days later), the animals were reinjected with serotonin solution as before and were monitored for 2 hours. No spawning occurred. Warm shock was then attempted as before. After warming, water in the spawning pan was allowed to cool. Several individuals spawned within minutes of each other, beginning approximately 1.5 hours after warming began. It was not possible to determine how many animals had spawned due to the turbidity created by spawning. The temperature in the pan was  $28.3^{\circ}\text{C}$  when the animals spawned.

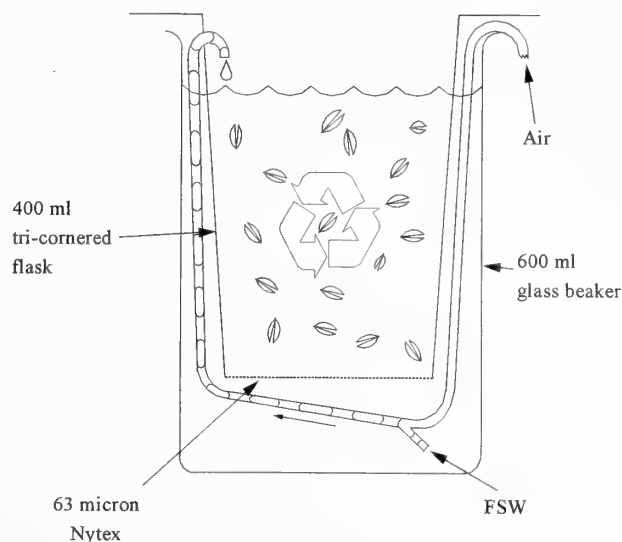


Figure 1. Diagram of Airlifted-Droplet Stirrer vessel. Droplets are lifted from bottom of vessel by air passing Y-tube at bottom. Larvae are kept well mixed within vessel because (1) surface tension is disturbed which prevents positively buoyant larvae from aggregating at the surface, and (2) turbulence is created by circulation within the vessel.

### Larval Culture

Water was removed from the spawning pan, sieved through cheesecloth, and transferred into shallow glass bowls. A compound microscope was used to measure the largest dimension of 50 fertilized eggs removed from the spawning pan with a disposable pipette. The fertilized eggs that remained were then removed from the spawning pan using a pipette under a dissecting microscope and placed in 10 sterilized 500 mL glass beakers containing  $0.2\ \mu\text{m}$  FSW. Filtered seawater was changed three times to decrease the concentration of pathogens. Streptomycin sulfate and penicillin-G sodium were added to the larval culture media as antibiotics, initially at  $50\ \mu\text{g mL}^{-1}$  for each, but concentrations were doubled 3 days after spawning due to high larval mortality (estimated at  $> 75\%$ ). Developmental stages of 20 haphazardly chosen live larvae from three randomly chosen beakers were noted at 1 to 3 hour intervals for the first 48 hours. After that, at least 200 larvae were placed in each of 12 600 mL culture vessels. The culture vessels were continually stirred using air to lift water from the bottom of the vessel and return it at the surface (Figure 1). These larval culture vessels were designed by Michael Hadfield, and are described by Strathmann (1987:16). This design reduces the number of larvae stuck at the water's surface. Twenty haphazardly chosen larvae were removed from three randomly chosen culture vessels every 2 to 3 days for measurement of the largest dimension and determination of developmental stage. This method of determining larval size and developmental stage was used to minimize in-

Table 1

Numerical codes of developmental stages for descriptive statistical analysis.

Larval stage	Numerical code
Fertilized egg	0
First Cleavage (includes all cleavage stages)	1
Blastula	2
Gastrula	3
Trochophore	4
Prodissoconch I (Straight-Hinged)	5
Prodissoconch II (Umbo Veliger)	6
Pediveliger	7

dividual larval handling. The number of culture vessels decreased as larvae died or settled and larvae from different culture vessels were combined. Developmental stages were numerically coded for descriptive statistical analysis (see Table 1). Salinity in the vessels was approximately 34, and the temperature varied between 22° and 24°C. Vessel seawater was changed every 1 to 2 days, and dead or abnormal larvae were removed.

#### Larval Feeding and Algal Culture

Larvae were fed dilute suspensions of *Isochrysis galbana* and *Skeletonema costatum* (log-growth stage) beginning when larvae reached the first feeding stage (straight-hinged stage) 24 hours after fertilization. The concentrations of algae in the feeding suspensions were not determined. However, it was apparent that the larvae were feeding because visual inspections showed their guts were full of algae. Enough algae were added to the vessels to keep larvae guts full throughout the culture period. *Isochrysis galbana* and *S. costatum* were cultured in autoclaved f/2 medium-enriched seawater (Bidwell & Spotte, 1985:305) under continuous illumination by a cool white fluorescent light in aerated culture flasks.

#### Settlement

Shell fragments of adult *S. tenebrosus* were placed into three randomly chosen larval culture vessels after the first pediveligers appeared in the samples in an attempt to induce settlement. The shell fragments were obtained from adults that were crushed minutes before the settlement experiments began. Larvae were not counted in settlement vessels; instead, the cumulative number of settlers was counted on the days that developmental stage and size data were recorded. Cumulative counts of settlement were approximate due to the complex surface topography of shell fragments. Shell fragments were not added to the remaining culture vessels in order to determine the ability of *S. tenebrosus* to remain competent for prolonged larval

periods. Shell fragments were finally added to these vessels 2 days before the end of the culture experiment. Larvae were cultured for a total of 60 days after fertilization.

#### Field Temperature Record

Warming is a spawning cue for many species of bivalves (Strathmann, 1987) and pectinaceans specifically (Cragg & Crisp, 1991). Therefore, an oceanic temperature record was needed to determine if a warming event occurred when *S. tenebrosus* spawned in Mamala Bay between 10 August and 21 September. However, no temperature record was available for the Bay. Alternatively, a temperature record from a C-MAN (Coastal-Marine Automated Network) buoy 51026 (21°21'06"N, 156°55'54"W), located 17 km north of Molokai and ~ 90 km from Mamala Bay, was obtained from the National Oceanographic Data Center's online archive. The buoy temperature record provides a useful indicator of regional warming events lasting several days for the region that includes Molokai and Oahu. The NODC C-MAN buoy 51026 temperature record contains hourly data. These data were resampled at a daily frequency after lowpass filtering with an eighth order Chebyshev type I lowpass filter to remove short-term (e.g., tidal period) variations.

#### Data Analysis

Statistical testing of growth and developmental data was problematic since different larvae from different culture vessels were measured over the course of the experiment (this was done to minimize handling of individual larvae). Therefore, it was necessary to assume that larvae from all culture vessels formed one population. In order to lump data among culture vessels for time series of size and development, it was necessary to test for differences in these parameters among culture vessels. The null hypothesis was that the sizes and developmental stages of larvae were not different among culture vessels each day that measurements were conducted. This hypothesis was tested using the non-parametric Kruskal-Wallis test (Sokal & Rohlf, 1969). No significant differences among culture vessels for either size or developmental stage were observed ( $\alpha = 0.05$ ). Therefore, larval size and developmental stage data were pooled among culture vessels within each day that measurements were conducted. The means and standard deviations for each parameter were then plotted as a function of time.

## RESULTS

#### Natural Spawning in Mamala Bay

A warming event occurred in late August and early September (Figure 2), which may have triggered *S. tenebrosus* in Mamala Bay to spawn sometime between 10 August and 21 September 1996, when adults were collected. Water temperatures increased from 24.4°C to 26.4°C



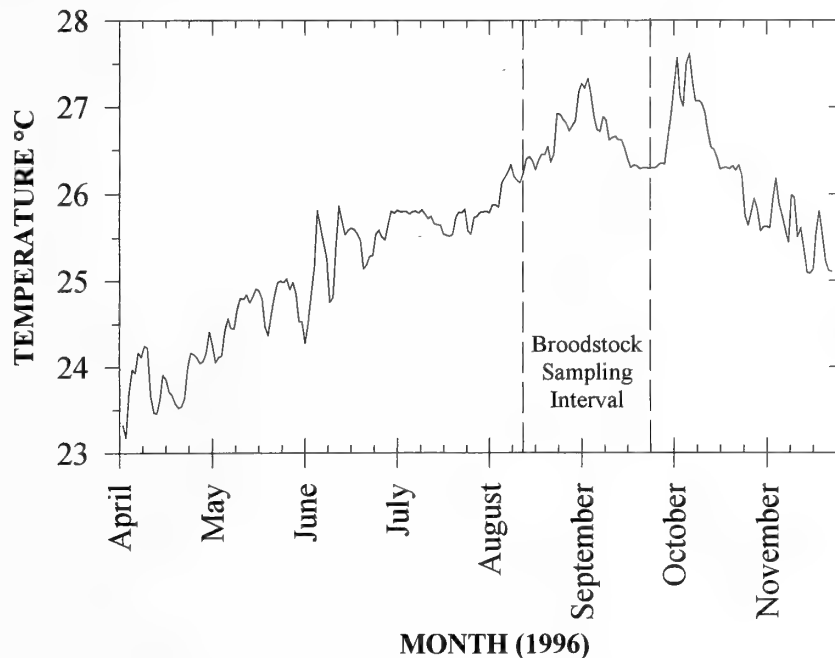


Figure 2. Temperature data from C-MAN buoy 51026 (17 km north of Molokai). Dotted lines indicate broodstock sampling dates.

from early June to 10 August. Temperatures then increased at a faster rate from 26.4°C, on 10 August, to a maximum of 28.5°C, on 31 August. Water temperatures decreased after the warming event at a rate similar to the rate of increase prior to the 31 August maximum; the temperature cooled to 26.3°C by 21 September. A second temperature maximum of 28.4°C was observed on 4 October.

### Larval Development and Growth

Larvae were not counted during the culture period. However, it is estimated that at least 75% of larvae died within the first 3 days. Antibiotic concentrations were doubled on the fourth day, after which larval mortality appeared to decrease dramatically. It is not clear whether mortality rates decreased due to increased antibiotic concentrations or decreased larval density. Few mortalities were observed after larvae reached the umbone stage. Mortality rates appeared even lower after larvae reached the pediveliger stage.

Larval development stages are plotted as a function of time in Figure 3. Figure 3 is a semi-log plot because the first week of development—during which *S. tenebrosus* larvae developed through five stages—is emphasized relative to the remaining 7-week culture period—when development progressed through only two stages. (The time periods below refer to time after fertilization.) First cleavage occurred within the first hour, and cleavage stages were observed for up to 6 hours. Blastula stages were observed within 4 to 8 hours. Gastrulation was first ob-

served at 6 hours (5.0% of larvae), and gastrulae were observed until 17 hours (5.0%). Trochophores first appeared within 11 hours (3.3%) and were present in the samples for 40 hours (5.0%). Straight-hinged larvae were first observed in the 21st hour (11.7%), and 8.3% of sampled larvae were straight-hinged on day 10. Umbone-stage larvae appeared within 6 days and were observed until 42 days after fertilization (5.0%). Pediveligers first appeared 12 days after fertilization, and more than 100 were still alive when culture was ended on day 60.

Figure 4 illustrates sample means and standard deviations of larval size (open circles) and developmental stages (filled triangles) as a function of time after fertilization. Three larval growth rate periods were observed. The first period included development from the fertilized egg to the trochophore stage when growth was negative. Mean size decreased from 64.3 to 61.9  $\mu\text{m}$  during this period. The second growth period occurred during development from trochophore to the pediveliger stage. The growth rate during this period (11.4  $\mu\text{m day}^{-1}$ ) was the greatest observed over the time series. The average growth rate of pediveliger larvae, the third larval growth period, decreased to 1.0  $\mu\text{m day}^{-1}$ , and growth was asymptotic. An asymptote of  $\sim 320 \mu\text{m}$  was calculated from the fit of a cubic regression of size as a function of time.

### Larval Behavior

Larval behavior varied during the culture period. Trochophore through umbone larvae were active swimmers, and were seldom at the bottom of culture vessels

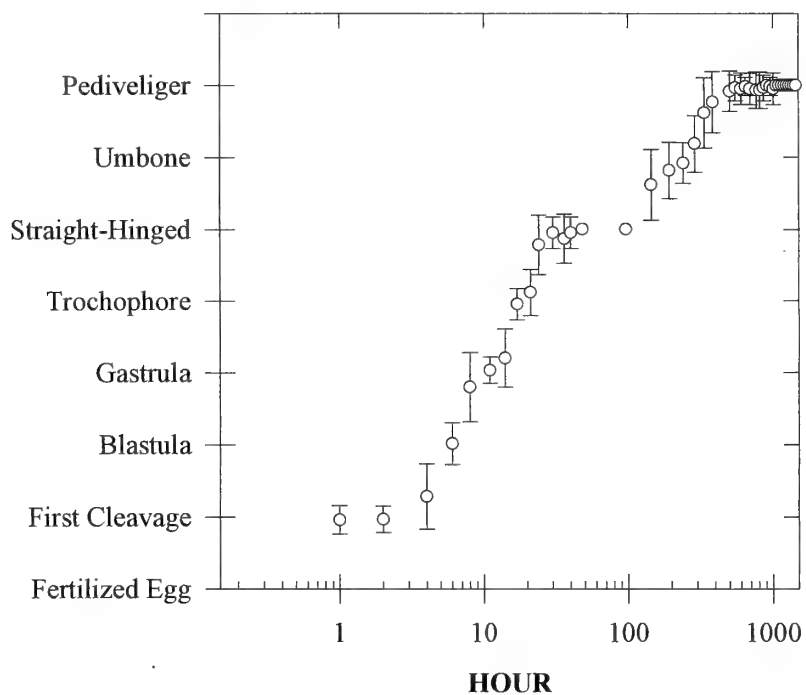


Figure 3. Larval developmental stage as a function of time after fertilization. First week of development is easier to visualize on semi-log plot. Larval developmental stages were numerically coded (see Table 1). Time of first observed settlement is indicated. Error bars are one standard deviation of individuals pooled among larval culture vessels.

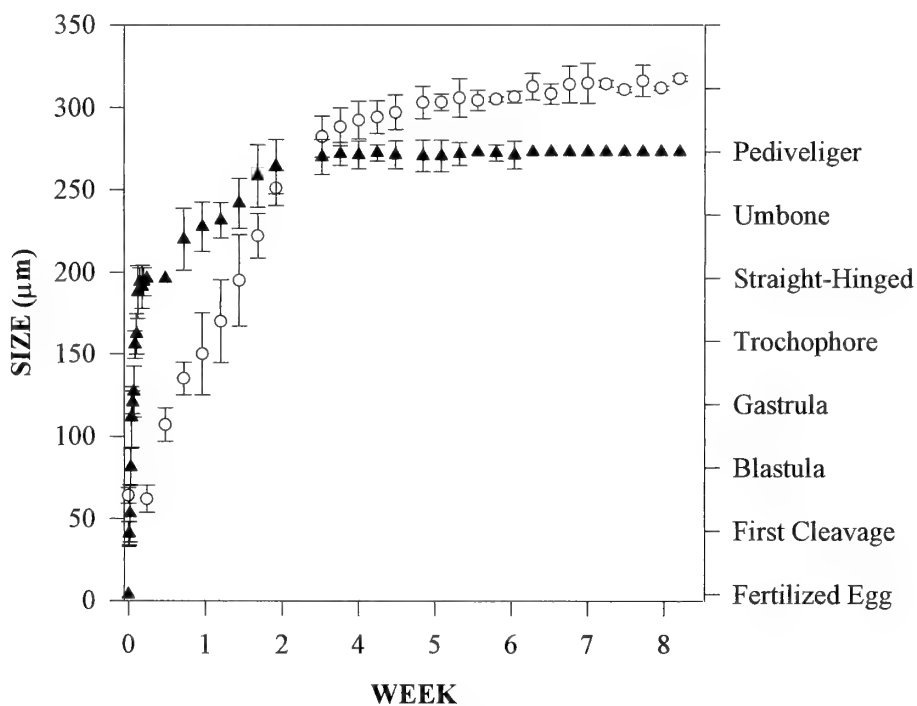


Figure 4. Larval size (open circles) and developmental stage (filled triangles) as a function of time after fertilization. Time of first settlement is indicated. Error bars are one standard deviation of individual larvae pooled among culture vessels.

except when disturbed. In contrast, pediveligers alternated between swimming and crawling on the bottom. Periods between planktonic excursions increased through time. Pediveligers still fed while on the bottom by orienting themselves velar-side-up and creating feeding currents with their cilia. The larger pediveligers appeared to sink faster than smaller larvae, and spent most of their time on the bottom of the culture vessels. The feet of these larvae were so large (relative to velum) that they appeared to interfere with swimming.

### Larval Settlement

Pre-soaked adult shell fragments were added to three culture vessels when the first pediveligers were observed (day 12). Settlement was first observed on day 21 (12 December) when 148 spat were counted. The cumulative number of spat increased to 180 by day 23, and decreased to 163 on day 25. Mortality due to handling was the most likely cause of the decrease. The number of settlers further declined after day 25 in the settlement vessels.

Shell fragments were added to the vessels which had never had settlement substrate added on day 58, to determine if the pediveligers were still capable of settling. No settlement was observed when larval culture was terminated on day 60 even though the larvae appeared healthy, their guts were full of algae, and they were still capable of swimming.

### DISCUSSION

The population of *S. tenebrosus* on the Waikiki artificial reef spawned between 10 August and 21 September 1996. It is likely that other populations of *S. tenebrosus* in Māhala Bay spawned during this period because the gonads of all the animals sampled from other areas in the Bay on 23 September were empty. Temperature is therefore circumstantially supported as a field spawning cue for *S. tenebrosus* since warming and spawning occurred within the same 3-week period. Many other pectinaceans have been observed to spawn during summer/fall annual temperature maxima (Bonardelli et al., 1996; Tammi & Turner, 1997; Villalejo-Fuerte & Garcia-Dominquez, 1998; Baqueiro & Aldana, 2000).

The spawning cue that induced adults to spawn in the lab is unclear since the animals were both injected with serotonin, and warmed. Serotonin injections did not induce spawning within 2 hours, but the serotonin injections in combination with warming may have triggered spawning. However, warming alone may also have triggered spawning. Warming is supported as a spawning cue in the lab because, in this study, natural populations spawned during a warm event, and other pectinaceans spawn in response to warming in the lab (e.g., Monsalvo-Spencer et al., 1997). As a caveat, the results of other lab spawning studies suggest that cold-shock is an effective spawning cue for some tropical pectinaceans (Velez et al.,

1990; Chaitanawisuti & Menasveta, 1992); cold-shock was not attempted in this study. Further work is needed to determine if warming is a consistent spawning cue for *S. tenebrosus* in the lab and for natural populations.

Larval development of *S. tenebrosus* from fertilization to the straight-hinged stage (first feeding stage) occurs within 24 hours. This rate of development during early stages is similar to that reported for other species of tropical pectinaceans (Bellolio et al., 1993; Chaitanawisuti & Menasveta, 1992) and faster than some temperate pectinaceans (Strathmann, 1987). The period that *S. tenebrosus* takes to develop to the pediveliger stage is shorter (by a few days) than other tropical pectinaceans (Belda & Del Norte, 1988) and up to 2 weeks shorter than temperate pectinaceans (Beaumont & Budd, 1982; Strathmann, 1987).

The results of this study suggest that the minimum precompetent period for larvae of *S. tenebrosus*, cultured at 22.0° to 24.0°C, is approximately 21 days. The utility of this laboratory-derived period to estimate natural precompetent periods is arguable, given that temperature and food availability in the field is variable. The precompetent period is likely to decrease with increasing temperature, and increase with decreasing food availability. The seasonal range of Oahu surface coastal water is 22.0° to 28.5°C (personal observation), and veligers of *S. tenebrosus* occur in the plankton year-round (personal observation). Therefore, *in-situ* precompetent periods may be less than 21 days during spring, summer, and fall, when the temperature is greater than 24.5°C, and longer during winter when the temperature is lower. It is likely that natural populations of larvae are exposed to lower food concentrations than the larvae cultured in this study. Therefore, the precompetent period of natural larvae may be longer than 21 days any time of year.

More than 100 pediveligers that never had substrate added to their vessels survived for the entire 60 day culture period. These pediveligers appeared healthy when culture was discontinued, and probably would have survived longer. The effect of delayed settlement on dispersion is questionable since older and larger pediveligers appeared to swim for shorter periods with increasing culture period. Events such as periods of large swell may increase the dispersion of long-lived, bottom-dwelling pediveligers through resuspension, which would extend the period that these larvae are subject to dispersal. The fact that the larvae that were prevented from settling until day 58 did not immediately settle when substrate was added to their culture vessels suggests that the period that larvae can delay settlement and successfully settle is limited to less than one season.

The results of this study indicate that *S. tenebrosus* is capable of completing its reproductive cycle in the lab within 60 days. Therefore, natural populations of *S. tenebrosus* in Hawaii likely reproduce more than once per year. A 60-day reproductive cycle for pectinaceans is not

unusual since the reproductive cycle of *Argopecten ventricosus*, a hermaphroditic pectinacean, was observed to be as short as 27 days in the lab (Monsalvo-Spencer et al., 1997). Natural populations of many other pectinaceans spawn more than once per year (Baquero & Aldana, 2000).

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## Mass Exhumation and Deposition of *Mulinia lateralis* (Bivalvia: Mactridae) on an Intertidal Beach, St. Catherines Island, Georgia, USA

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**Abstract.** Episodic events which affect populations of marine invertebrate species are rarely documented. We report the catastrophic mass exhumation and deposition of a large aggregation of adult bivalves (*Mulinia lateralis* [Say, 1822]) to a suboptimal habitat on a sandy intertidal beach of St. Catherines Island, Georgia, USA. The displaced population impacted a large area (7000 m<sup>2</sup>) of the beach and consisted of similar-sized clams (~13 mm mean shell length). We suggest that the exhumation could have been a result of storm-induced shear stress, an hypoxic event, or other environmental stress on the individuals. Events of this type could have important implications for population dynamics and cohort distribution, fisheries predictions and harvests, and interpretation of fossil assemblages.

### INTRODUCTION

On 4 October 1993 we observed a large patch of *Mulinia lateralis* (Say, 1822), the dwarf surfclam, in the intertidal zone on South Beach, near Flag Pond, St. Catherines Island, Georgia (Figures 1, 2). This was a notable occurrence because most adult infaunal bivalves are sedentary, moving long distances only as larvae or stochastically by rafting with eroded substrata, and because *Mulinia lateralis* are normally found subtidally.

*Mulinia lateralis* typically occurs in near-shore environments along the Atlantic and Gulf coasts of the United States and can be present subtidally in very dense infaunal aggregations. *Mulinia lateralis* can occur episodically and in very high densities (21,000 m<sup>-2</sup>) subtidally (Santos & Simon, 1980). Santos & Simon (1980) found that an ephemeral population of *M. lateralis* in Tampa Bay, Florida had an average density of approximately 5700 m<sup>-2</sup> when present. Montagna et al. (1993) reported a population in Laguna Madre, Texas with densities up to 800 m<sup>-2</sup> soon after recruitment in the spring, and low densities (< 100 m<sup>-2</sup>) for the majority of the year. Walker & Tenore (1984) found that the density varied with habitat in Wassaw Sound, Georgia. Populations with the highest average density were in sandy mud (10,161 m<sup>-2</sup>), whereas mud and sand habitats had lower densities (277 m<sup>-2</sup> and

263 m<sup>-2</sup>, respectively), but all population densities fluctuated widely. *Mulinia lateralis* populations have not been reported occurring intertidally in such dense live aggregations as we report, and apparently this aggregation was exhumed and deposited.

### OBSERVATIONS

The site of the *Mulinia lateralis* accumulation, South Beach, is a medium-energy (silty-sand) beach on the seaward side of St. Catherines Island. St. Catherines Island is a relatively pristine environment as there is little human activity on the island except in a research and conservation compound on the north-west (leeward) portion. Mean tidal amplitude is approximately 2.5 m. High tides were increasing toward a maximum, from +2.1 to +2.6 m mean low water at the time of observation, and this condition had been present during the 5 days preceding our observations. There had been no significant rainfall since 27 September 1993 when 0.2 cm fell (as recorded on Sapelo Island, Georgia). Wind velocity recorded on Sapelo Island had remained below 10 m/sec for the month prior to our observation and reached a velocity of 8.36 m/sec on 30 September 1993.

To quantify the extent of the exhumed population in the intertidal zone, we sampled at ebb tide along a tran-

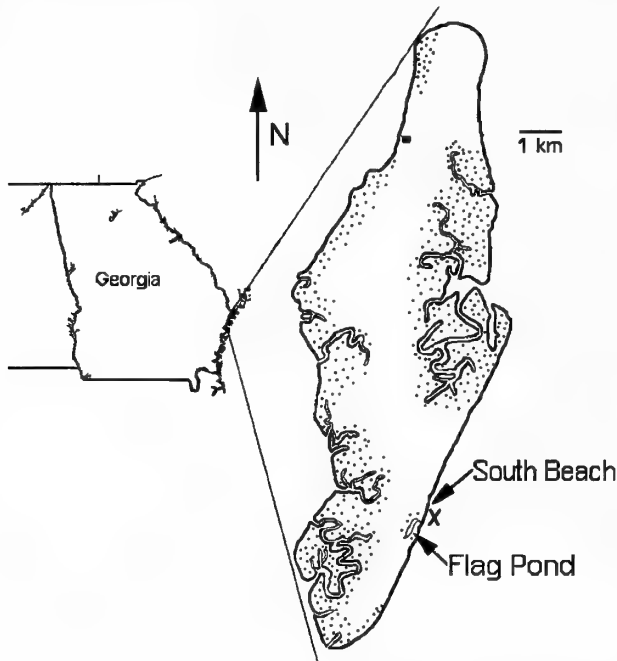


Figure 1. Diagrammatic map of St. Catherines Island, Georgia. Salt marsh is indicated by stippling. The study site is indicated by an X.

sect extending from the wrack line, approximately 80 m landward of the low tide line, to a tide level present 1 hour before maximum low tide. We used circular quadrats of 30 cm diameter (area = 706.5 cm<sup>2</sup>) to sample at 2 meter intervals along the transect. All live and dead clams present to a depth of 7 cm were collected. The number of live and dead clams within each quadrat was counted. Where clams were present on the surface, as well as buried, the ratio of surface to subsurface clams was noted.

The exhumed clams covered a large area of the beach (Figure 2). The surface aggregation extended approximately 17 m north to south and 14.5 m east to west (246.5 m<sup>2</sup>), whereas the sub-surface accumulation was much larger and extended approximately 87 m north to south from the high intertidal into the subtidal zone (7000 m<sup>2</sup>). A large, but unquantified, traction load of live and dead clams also was present in the outgoing tidal swash zone.

Clams occurred on the surface midway between the swash zone and the wrack line, 24–72 m seaward from the high intertidal zone (Figure 3). Within this zone, the greatest density of exhumed clams occurred between 42 and 52 m from the high intertidal zone. The highest density of live clams occurred at 46 m (23,227 live clams m<sup>-2</sup>; Figure 3). Dead shells were much less abundant, but their distribution paralleled that of the live clams, possibly indicating passive transport or post-depositional mortality. Live clams composed 78.7% of all shells collected. Between 42 and 52 m from the upper intertidal zone, the surface shells (75%) outnumbered the buried shells (Fig-



Figure 2. *Mulinia lateralis* exposed on South Beach, St. Catherines Island, Georgia on 4 October 1993. The infaunal population extends from high to low tide lines, while the surface clams are aggregated between 42 and 52 meters from the high tide line. *Anadara ovalis* and *Busycon* species are also present. Scale bar represents 1 meter.

ure 4), but there were no differences in the proportions of dead and live clams in these samples. The mean length of all clams was  $12.84 \pm 1.17$  mm (Figure 5) with no significant ( $P < 0.001$ ) difference between dead and live clams. The majority of live clams examined were sexually mature with ripe gonads.

#### ACCUMULATION ORIGIN

The mass exhumation of *Mulinia lateralis* reported here was notable because of the limited spatial distribution and because of the very high density of clams involved. Levinton (1970) reported large aggregations of dead valves of this species in Long Island Sound and Narragansett Bay, Rhode Island, and discussed the significance of such dense death assemblages for the fossil record. He suggested that those assemblages were the result of post-mortem transport. Other bivalves, notably the surf clam *Spisula solidissima* (Dillwyn, 1817), were observed washed up on New Jersey beaches near their subtidal populations; however, the majority observed during this event were dead or dying (Boyajian & Thayer, 1995). The authors described a storm-deposit of surfclams, and suggested mechanisms of exhumation and deposition, including the hypothesis that storms could remove overlying sediment, increasing the likelihood of subsequent population excavation and size-selective excavation and deposition. Rees et al. (1977) also noted storm-induced strandings of several bivalve species along the coast of North Wales. They stated that wave activity could be a factor in the maintenance of soft bottom benthic associations in near-shore waters.

Although no storms had occurred along the Georgia coast in the month prior to the exhumation event, large waves remain the likely mechanism transporting these

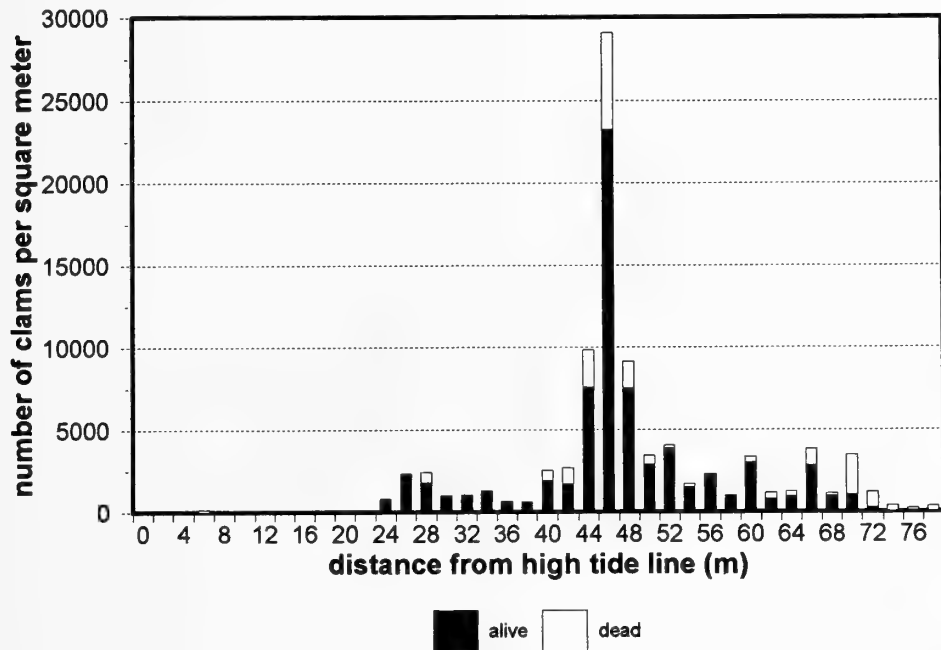


Figure 3. Frequency distribution of *Mulinia lateralis* occurring in the intertidal zone of South Beach, St. Catherines Island, Georgia, on 4 October 1993.

adult megafaunal clams so high into the intertidal zone. Palmer (1988) discussed the importance of passive transport of meiofaunal species and concluded that for such small organisms it is a fairly important mechanism of dispersal of both adults and juveniles. Passive wave-in-

duced movement of any organism involves shear stress, and the larger the organism, the higher the shear stress needed to initiate movement (i.e., erosion) (Denny, 1988). Therefore, a relatively large shear stress, present in large waves or in storm-induced seas, was likely needed to lift

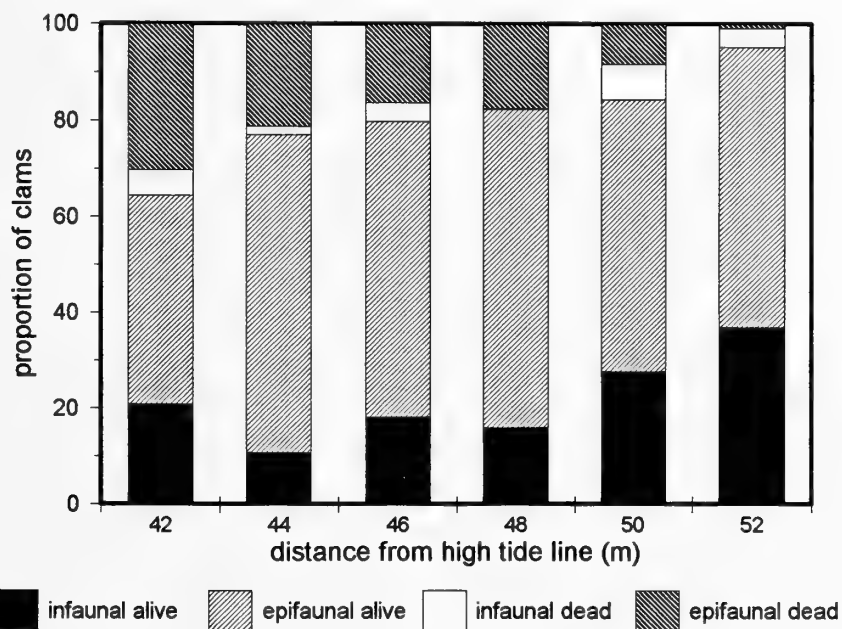


Figure 4. Percent contribution of dead and live *Mulinia lateralis* to surface and infaunal populations occurring in the intertidal zone of South Beach, St. Catherines Island, Georgia, on 4 October 1993.



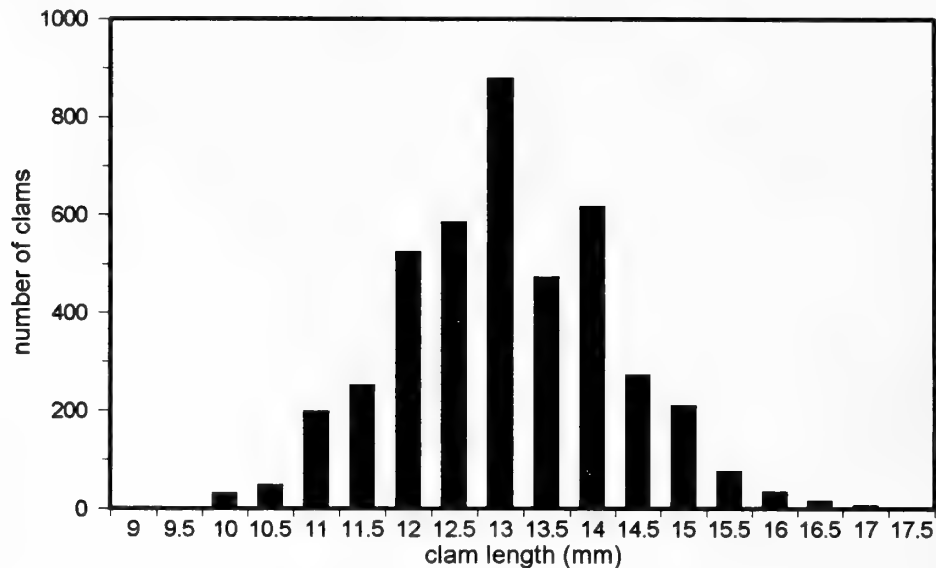


Figure 5. Length-frequency distribution of *Mulinia lateralis* occurring in the intertidal zone of South Beach, St. Catherines Island, Georgia, on 4 October 1993.

and initiate movement of these fairly large clams. Although tidal energy represents a potential source of movement of the clams, the tide prior to the exhumation event was not unusual in magnitude. Other mollusks, including the blood ark (*Anadara ovalis* [Bruguère, 1789]) and whelk species (*Busycon* species), which were much larger (5+ cm length) than *Mulinia lateralis* occurred in patches along the beach on the day of observation, possibly indicating community-wide disturbance, rather than a monospecific disturbance.

*Mulinia lateralis* has relatively short siphons that require it to remain near the surface to feed (Chalermwat et al., 1991). Therefore, passing of a shrimp otter trawl net over the population (commercial shrimp trawling is important in Georgia, especially during summer months, and occurs frequently in the ocean waters within sight of St. Catherines Island beaches) could facilitate the excavation of large numbers of individuals. Exposure on the sediment surface, combined with the strong tidal flow characteristic of the region or wind-driven mixing, could transport the clams into intertidal areas and deposit them. There were many *M. lateralis* still in suspension and buried just beneath the surface in the swash zone (approximately 1400 clams m<sup>-2</sup>), indicating that the depositional event may still have been occurring, or that the population was being actively reworked at the time of observation.

Bivalves will often move close to or onto the surface when stressed by extremes in environmental conditions, such as low salinity or hypoxic events, possibly enhancing the likelihood of exhumation and transport of a population (Cleveland, 1991; Richardson et al., 1993). We have no records of subtidal environmental parameters,

such as salinity and temperature, for this area, and therefore can only speculate as to what caused the observed phenomenon. It seems unlikely that these clams were transported a long distance before being deposited and were probably from an area relatively nearshore in the vicinity of South Beach. *Mulinia lateralis* typically inhabits sandy-mud substrata (Walker & Tenore, 1984), which are abundant in areas around St. Catherines Island. Most likely, a cojacent population was exhumed and displaced.

#### EFFECT ON POPULATION DYNAMICS

Events similar to the one observed and described could effectively entrain an entire population of clams and move it to a new site. If the exhumation is extensive, the entire population could be deposited onshore, resulting in high mortality by the stress of dislodgment, desiccation, and extreme temperature. *Mulinia lateralis* is an opportunistic species that colonizes areas quickly (Levinton, 1970), and therefore, exhumation of this type, prior to a major recruitment event, could have short-lived effects on the overall population dynamics.

Previous observations of bivalve movement have shown that some large adults, such as the northern quahog *Mercenaria mercenaria* (Linnaeus, 1758), can be entrained in high energy waters leading to an adjunct mode of dispersal beyond larval propagules (Prezant et al., 1990; Rollins et al., 1992; Boyajian & Thayer, 1995). Also, vagrant bivalves, such as *Donax* species, move regularly across a habitat (Ansell & Truman, 1973). Passive transport resulting in colonization of a habitat can be an important mechanism for population dispersal and estab-

ishment for opportunistic species (Emerson & Grant, 1991). Hydrodynamic factors are also known to be important in the dispersal of larval bivalves and can result in patchy recruitment events. Bedload transport of juvenile soft-shell clams *Mya arenaria* Linnaeus, 1758, can affect population dynamics by immigration of large aggregations into underutilized habitats (Emerson & Grant, 1991). The common cockle *Cerastoderma edule* Linnaeus, 1758, lives in the top few centimeters of sediment, and the combined stressful effects of waves, currents, and burial have been shown to cause the emergence of large numbers of these clams, thereby enhancing the likelihood of their passive entrainment and transport (Richardson et al., 1993). Scallops are notorious for their locomotory ability whereby adults can swim horizontally and migrate to new habitats (Carsen et al., 1995). Juveniles, however, swim vertically and are then advected horizontally by currents and possibly moved into more hospitable habitats (Carsen et al., 1995). The accumulation of *Mulinia lateralis* described here was composed of adult individuals, providing evidence of the importance of adult dispersal in bivalve population dynamics.

### IMPLICATIONS

Observation and reporting of unexpected ecological phenomena such as the one described here can provide valuable information about population ecology and life history of organisms, as well as information useful for interpretation of fossil assemblages (Boyajian & Thayer, 1995). Although population studies and transplantation experiments provide useful information about a species, unrecorded episodic events can produce effects that could subsequently appear in a population and lead to erroneous conclusions regarding range and cohort dynamics. For example, the size-selective mass exhumation of a portion of a bivalve population could leave a population with the length frequency skewed toward older (or younger) individuals. Future age-class analyses could record this as a low recruitment event, when, in fact, recruitment was normal for the size classes affected by the exhumation.

Interpretation of fossil assemblages could be biased by deposition of large numbers of live animals as well as dead shells (Levinton, 1970; Rollins et al., 1992; Aguirre & Farinati, 1999; Walker & Goldstein, 1999). Although we do not have any information on the post-depositional fate of this assemblage, we do know that the sandy intertidal beach is not ideal habitat for *Mulinia lateralis* (Levinton, 1970). Morris & Rollins (1977) described some life-positioned bivalve fossil assemblages on St. Catherine's Island. Interpretation of such fossil assemblages must take into account the history of the assemblage prior to death as well as that after death (taphonomy). The majority of these *M. lateralis* were alive, but their condition could have been weakened by the stresses from exhumation, transport, deposition, and desiccation

in the intertidal zone in such high densities. If this assemblage remained intact and was buried on the beach, it could be misinterpreted as an in situ population. Alternatively, the assemblage could be interpreted as a transported death assemblage. Some of the live clams were in life position and could be misinterpreted as having recruited to this habitat as juveniles rather than adults (Rollins & West, 1997; West et al., 1990). There are many ways that this event could be interpreted that could lead to rational but erroneous conclusions. Documentation of these events can provide useful information about a species, community or fossil assemblage, and have bearing on shellfisheries' predictions, yields, and harvests.

**Acknowledgments.** Thanks to Mr. Royce Hayes, Superintendent of St. Catherine's Island, for his on-site support and extensive knowledge of the Island. We are grateful for grant support from the St. Catherine's Island Foundation, Incorporated, administered by the American Museum of Natural History. We would also like to thank the University of Georgia Marine Institute on Sapelo Island for access to climatological data.

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## The Natural Diet of the Argentinean Endemic Snail *Chilina parchappii* (Basommatophora: Chiliniidae) and Two Other Coexisting Pulmonate Gastropods

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**Abstract.** In this paper we study the natural diet of *Chilina parchappii* (d'Orbigny) (Chiliniidae), *Biomphalaria peregrina* (d'Orbigny) (Planorbidae), and *Physa venustula* Gould (Physidae) in an artificial canal in the Province of Buenos Aires, Argentina. The close similarity between the organic particle composition of the sediment and the crop contents of the three species studied suggests they are basically non-selective feeders. The food composition does not differ from the *Aufwuchs* composition. Diatoms and detritus particles are the main food items for the three species. Although there is a high degree of diet overlap among the three species, a principal component analysis revealed interspecific differences in diet. *Biomphalaria peregrina* is more detritivorous; *Chilina parchappii* ingests more diatoms; and *Physa venustula* consumes more non-filamentous algae. Experimental analysis of the ingestion, egestion, and assimilation rates, and the assimilation efficiency suggests that the endemic *Chilina parchappii* is subject to a probable risk of competition in a case of food shortage. However, competition among snails is highly improbable in the present area of sympatry, i.e., the lower basin of the Napostá Grande stream, which is rich in detritus.

### INTRODUCTION

Periphyton and detritus particles are almost universal food items for freshwater gastropods. Although some selection against or for specific items can be found, pulmonates are predominantly non-selective feeders (Hunter, 1980; Madsen, 1992; Brendelberger, 1995, 1997).

Selective grazing by freshwater gastropods has, however, been proposed as a cause of shifts in the succession of the benthic algae (Tuchman & Stevenson, 1991) or changes in periphyton composition (McCollum et al., 1998). Whereas detritus was by far the most common item in the gut of 20 snail species in England (Reavell, 1980), *Planorbis vortex* (Linnaeus) specifically selected against detritus and for diatoms (Lodge, 1986). *Ancylus fluviatilis* (Müller) also preferred diatoms and other periphytic algae, and did not eat detritus or fungal hyphae (Calow, 1973a, b). Trophic strategies are therefore variable among the freshwater snails, according to environmental and functional conditions.

The most abundant species of pulmonate snail in the lower basin of the Napostá Grande stream (Buenos Aires Province, Argentina) are the recently introduced *Physa venustula* Gould, 1847 (Physidae), the native *Chilina parchappii* (d'Orbigny, 1835) (Chiliniidae), and *Biomphalaria peregrina* (d'Orbigny, 1835) (Planorbidae).

The natural diets of these three South American species have not been studied previously. Some extrapolations are possible from the literature showing that the Planorbidae

and Physidae show great ecological uniformity (Calow, 1973b; Hunter, 1980; Reavell, 1980; Kesler et al., 1986; Underwood & Thomas, 1990; Madsen, 1992; Carman & Guckert, 1994), but nothing is known at present about the feeding habits of the South American endemic family Chiliniidae. Species of Chiliniidae have been deemed to feed only on diatoms (Brace, 1983; Bosnia et al., 1990).

In this paper we study the natural diet of *Chilina parchappii*, *Biomphalaria peregrina*, and *Physa venustula* with the aim of determining whether they feed non-selectively and whether the diet of the invading species (*P. venustula*) overlaps those of the native species (*B. peregrina* and *C. parchappii*).

### MATERIALS AND METHODS

The sampling site is an artificial canal within Parque de Mayo, an urban park in Bahía Blanca city (38°44'S–62°00'W, Argentina). It is fed with water from the Napostá Grande stream. The selected portion of the canal is 70 m long and about 4 m wide, with a maximum depth of 0.60 m. The sediment is mostly sandy silt, with a high proportion of detritus. The macrophytes *Myriophyllum elatinoides* (L.), *Potamogeton striatus* Ruiz & Pavon, and *Chara contraria* A. Braun ex Kütz., form dense mats in the center of the canal during most of the year. The biomass of the former two species decreases dramatically in winter.

Sampling was performed on six dates from December 1992 to November 1993. Individuals of the three snail species were picked up by hand along the canal margins,

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and immediately killed by immersion in hot water. The soft parts of the snails were extracted in the laboratory and frozen at  $-20^{\circ}\text{C}$  for further analysis. Freezing was the best procedure to preserve the ingested algae, as revealed by preliminary trials of different preservation techniques (alcohol, formaldehyde, FAA, freezing); the alterations produced by chemicals made it difficult to recognize food items.

In winter (July), only individuals of *Chilina parchappii* were found in the canal, and their digestive tracts were empty, except for a small quantity of mineral particles retained in the stomachs. This sampling season was therefore excluded from the comparative analysis.

To detect possible ontogenetic differences in the diet we defined two disjunct size classes for each species, as follows: *Biomphalaria peregrina* (maximum diameter), young  $< 7$  mm, adult  $> 13$  mm; *Chilina parchappii* (shell length), young  $< 7$  mm, adult  $> 17$  mm; *Physa venustula* (shell length), young  $< 5$  mm, adult  $> 8.7$  mm.

The natural diets were analyzed by spreading the contents of the rear portion of the esophagus or crop in a drop of distilled water, prior to their observation under a compound microscope at a magnification of  $\times 400$ . We examined four to 14 snails for each species and date. The algae were identified to the generic or specific level, but were grouped into six groups for quantitative analyses: blue-green algae (BG), diatoms (Di), filamentous green algae (F), non-filamentous algae (unicellular, paucicellular, or colonial) (NF), detritus (De), and sand (S). The presence of these six items was recorded on 20 randomly selected microscope fields per individual.

The volume of the crop contents varied among and within the snail species as a consequence of body size and the degree of gut fullness. Therefore, for each individual snail, we determined the proportion of microscope fields where each kind of food was recorded in relation to the sum of all the fields with positive records:

$$p_i = n_i / \sum n_i$$

where  $p_i$  is the relative proportion of fields with food  $i$ , and  $n_i$  is the number of fields containing the food  $i$  (Kesler et al., 1986).

On the first sampling date we took simultaneous samples of sediment and periphyton to investigate possible selective feeding by comparing them with crop contents. The samples were taken from three different sites by scraping or sucking with a pipette the surface where the snails were adhering to the substrate.

The crop contents of 154 dissected snails were analyzed by principal components (PCA), using the covariance matrix as input (Orlóci & Kenkel, 1985), to determine possible seasonal and specific variations in the snail diets.

Laboratory experiments were performed to quantify the ingestion rate, egestion rate, and assimilation efficiency of the three snail species. Only adult snails were used

in these trials. Forty-eight previously weighed glass slides ( $75 \times 25 \times 1.2$  mm) were placed in a periphyton sampling box, and immersed in an artificial shallow pond. Three weeks later, when a substantial growth of *Aufwuchs* was evident, the slides were transferred to the laboratory and washed with demineralized tap water to remove loose material. Algae attached to the slides were mainly diatoms (mostly *Navicula* spp.) and the disk-shaped green *Coleochaete* sp. For every gastropod species, nine groups of three snails each (previously starved for 24 hr) were placed in Petri dishes 95 mm in diameter, filled with 50 ml of demineralized tap water. Six groups received two colonized slides as food; the remaining three groups were used as controls (without food). Twelve colonized slides were immersed in similar Petri dishes, without snails, as a reference of non-predated periphyton biomass.

Snails were allowed to feed for 4 hours before slides were removed, washed, dried at  $60^{\circ}\text{C}$  for 48 hr, and weighed to the nearest 0.1 mg. During the feeding tests, the feces produced by the snails (feeders and control) were collected with a Pasteur pipette. After removal of the slides, fecal collection was continued for an additional 4 hours. The snails were then killed by immersion in hot water and their soft parts were extracted from the shells. Feces and soft parts were dried at  $60^{\circ}\text{C}$  for 48 hr, and weighed to the nearest 0.1 mg.

To quantify ingestion, egestion, and assimilation rates we used the following formulae:

$$\text{Ingestion rate} \quad \text{IR} = \text{TI}/\text{DW}$$

$$\text{Egestion rate} \quad \text{ER} = \text{TF}/\text{DW}$$

$$\text{Assimilation rate} \quad \text{AR} = \text{IR} - \text{ER}$$

$$\text{Assimilation efficiency} \quad \text{AE} = (\text{TI} - \text{TF}) \cdot 100/\text{TI}$$

Total ingestion (TI) represents the total amount of food ingested (in mg), and is calculated as the difference between the dry periphyton weight on the non-predated and predated slides after the feeding period. Total feces (TF) represents fecal production (in mg); it was corrected by subtracting feces produced by control snails. DW is snail dry weight (in mg). Mean values of IR, ER, AR, and AE of the three species were compared by one-way ANOVA on the transformed data (log transformation for the rates; arcsine square root for the efficiency). The *a posteriori* multiple comparisons were made by Scheffé tests.

## RESULTS

Although the basic morphology of the digestive system is the same in the three species, there are some differences in the strength and degree of differentiation of the stomach region. *Chilina parchappii* shows a striking contrast between the relatively broad, almost black crop opening to a strongly muscular, bulbous, pearl pink gizzard. The crop is also well differentiated in *Biomphalaria peregrina*, but the gizzard is less muscular. The stomach

of *Physa* is morphologically simpler, uniformly grey colored, with no clear differentiation between crop and gizzard, the latter being thin-walled.

Figure 1 shows the temporal variation of the whole crop contents of the three species. No ontogenetic differences were detected for any snail species with respect to trophic preference (test  $t$ ,  $P > 0.05$  in all cases). Because of this, data from young and adult snails were grouped for the rest of the analysis.

All snail crops contained some mineral particles (sand) that probably aid in grinding the food (mainly the diatom frustules). The proportion of mineral particles was, however, significantly different among the snail species ( $F = 16.82$ ;  $df = 2, 152$ ;  $P < 0.0001$ ). An *a posteriori* Scheffé's test showed that *Biomphalaria peregrina* was the species with the highest proportion of ingested sand, whereas no significant difference in sand content was detected between *Physa venustula* and *Chilina parchappii*.

The main food particles were detritus and diatoms in the three species. The most frequent diatoms were in the genera *Achnanthes*, *Cocconeis*, *Epithemia*, *Fragilaria*, *Gomphonema*, *Gyrosigma*, *Navicula*, *Nitzschia*, *Rhoicosphenia*, *Surirella*, and *Synedra*; with a lesser frequency we recorded the diatoms *Asterionella*, *Amphora*, *Cymbella*, *Diatoma*, and *Pinnularia*. The filamentous green algae were represented by *Cladophora* spp. and *Oedogonium* spp. The non-filamentous algae were *Scenedesmus* (constant in all dissected esophagi), *Ancystrodesmus*, *Coelastrum*, *Crucigenia*, *Oocystis*, *Pediastrum*, *Tetraedron*, and *Euglena*. The blue-green algae were always represented by *Chroococcus* and *Merismopedia*, but the filamentous *Anabaena* and *Oscillatoria* were also present in a lesser proportion.

We found fragments of macrophyte leaves (specifically *Potamogeton striatus*) in only two (1.3%) of the 154 analyzed crops.

While radular teeth were seldom found in the crop contents of *Biomphalaria* and *Physa* (4.9% and 5.7% of the crops, respectively), 46% of the stomachs of *Chilina parchappii* contained radular teeth, mainly marginal teeth with highly eroded cusps.

We also recorded occasional animal remains, mostly chaetae of oligochaetes, some statoblasts of *Plumatella* sp. (Phylactolaemata), shells of newly hatched *Heleobia parchappii* (d'Orbigny) (Gastropoda: Hydrobiidae), fragmented rotifers, and microcrustacean appendages.

Figure 2 shows the relative abundance of the different food categories in the digestive tube of *Biomphalaria*, *Chilina*, and *Physa* (irrespective of the body size) as compared with the organic particles in the substrate samples from late spring. The composition of the crop contents in the three species showed a great similarity to the substrate composition. The only significant differences were due to a higher proportion of non-filamentous algae in the crops of *B. peregrina* and *P. venustula* ( $t = 3.68$ ,  $df = 23$ ,  $P$

$< 0.001$ , and  $t = 4.86$ ,  $df = 21$ ,  $P < 0.0001$ , respectively).

The results of PCA performed on the diets (sand excluded) are shown in Table 1 and Figure 3. The first component was highly positively correlated with the relative abundance of Di, and highly negatively correlated with NF. Snails whose diet included more Di relative to NF scored highly on this component. The second principal component was highly positively correlated with Di and highly negatively correlated with De. Stomachs with high content of Di relative to De scored highly on this component.

There was a high degree of diet overlap among the three species, but PCA still revealed interspecific variations. The mean principal component scores of the three groups differed significantly for PC1 and PC2 (one-way ANOVA test, Table 1). The multiple comparison (Scheffé test) revealed that *Biomphalaria peregrina* is more detritivorous, *Chilina parchappii* ingests more diatoms, and *Physa venustula* ingests more non-filamentous algae.

Some seasonal variations in the abundance of the different items could be detected (Figure 3). The isolated position of the autumn (April) samples of *Physa venustula* was mostly due to the high content of non-filamentous algae, represented in this case by *Euglena* spp., a kind of organism that never appeared in the other snail species or on other sampling dates.

In the laboratory experiments, *Physa venustula* showed the highest rates of ingestion and egestion ( $P < 0.05$ ), whereas *Biomphalaria peregrina* always had the second highest position (Figure 4). The values for *Chilina parchappii* were extremely low as an outcome of its peculiar behavior. *Physa* and *Biomphalaria* remained on the colonized artificial substrates most of the time during the feeding experiment, and browsed actively on the slides with the radula. *Chilina* instead crawled around and across the Petri dishes, with few buccal movements, even when they passed over the slides, leaving a large amount of mucus on the substrata.

Assimilation efficiencies ranged from 34% to 82%, with the lowest mean value achieved by *Physa venustula*.

## DISCUSSION

Many authors have shown that freshwater pulmonate snails are non-selective, microphagous animals. In this category are included, for example, several species in the genera *Lymnaea*, *Helisoma*, *Biomphalaria*, and *Bulinus* (Callow, 1970; Hunter, 1980; Baluku et al., 1987; Smith, 1989; Adam & Lewis, 1992; Madsen, 1992). The differences in the diet of snails living in different water bodies mainly reflect the variation in the composition of the *Aufwuchs*. Dillon & Davis (1991) even proposed using snail stomach contents as samples of the local diatom assemblages.

The close similarity between the organic particle com-

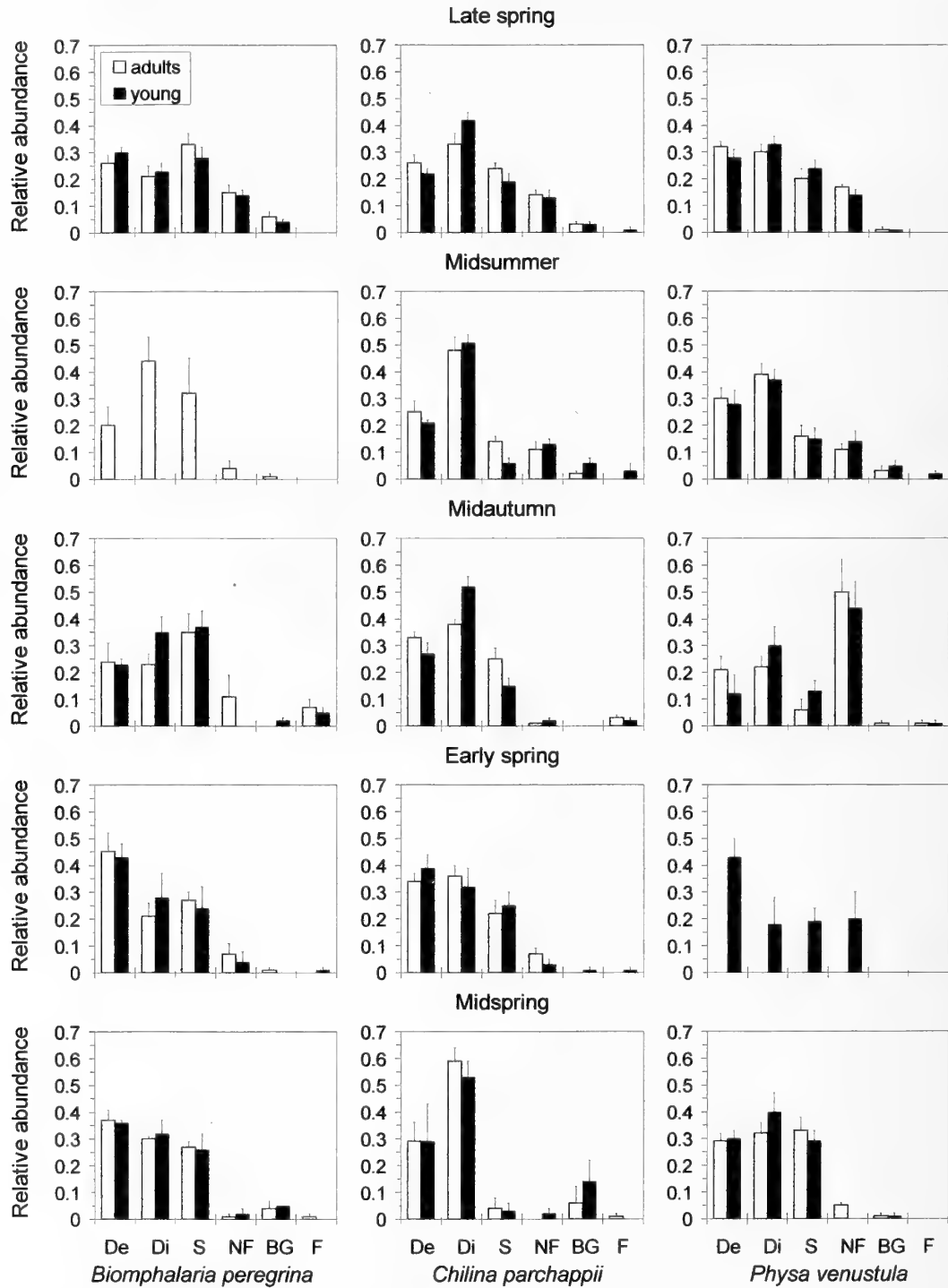


Figure 1. Crop content (mean  $\pm$  SE) of *Biomphalaria peregrina*, *Physa venustula*, and *Chilina parchappii* from an artificial canal in Buenos Aires province. De, detritus; Di, diatoms; S, sand; NF, non-filamentous algae; BG, blue-green algae; F, filamentous green algae.



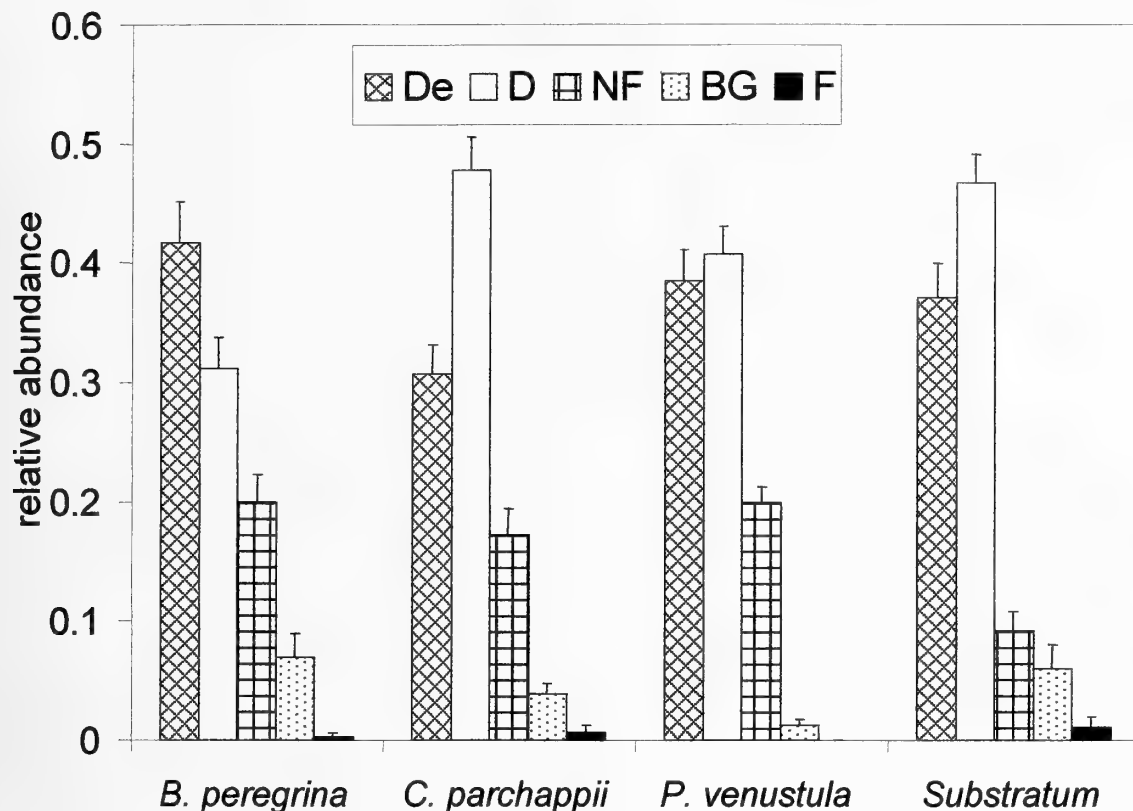


Figure 2. Crop content (mean + SE) of *Biomphalaria peregrina*, *Physa venustula*, and *Chilina parchappii* and the relative abundance of organic particles in the substrate samples from late spring. Abbreviations as in Figure 1.

position of the sediment and the crop contents of the three species studied suggests that they are basically non-selective feeders. When these animals feed on the same substrate, their diets are similar, and the diet composition does not differ from the *Aufwuchs* composition. The differences found among our snails are most probably due to the microdistribution of the patches of periphyton, resulting in a wide intraspecific variability.

Nevertheless, *Biomphalaria peregrina* exhibited a stronger tendency to eat more detritus than *Chilina* and *Physa*. This is consistent with the microdistributional information by Martín (1999) who stated that *B. peregrina* reaches its maximum abundance in the middle basin of the Napostá Grande stream, and that detritus affects its distribution. The middle basin receives organic contamination from a wide agricultural area, and effluent from the city. This portion of the stream was considered as mesosaprobic (Cazzaniga & Curino, 1987; Pettigrosso & Cazzaniga, 1987).

The lack of ontogenetic differences in the diet of the three species studied here is consistent with previous reports on *Biomphalaria pfeifferi*, *Helisoma duryi*, *Bulinus truncatus*, and *Bulinus forskalii* (Baluku et al., 1987; Madsen, 1992).

Many freshwater pulmonates carry sand particles in

their stomachs as a means of food grinding (Storey, 1970; Calow, 1973a; Reavell, 1980). Underwood & Thomas (1990) suggested that these mineral particles should also be a source of ions, micronutrients, and microorganisms. *Lymnaea peregra* shows low growth rates if its diet is devoid of mineral particles (Storey, 1970). *Biomphalaria glabrata* (Say) actively swallows sand and is able to select the size of the particles it retains in its digestive tube (Schmolder & Becker, 1990). Reavell (1980) found a very low proportion (and even absence) of sand grains in the diet of *Physa gyrina* (Say), indicating that the diameter of the mouth was a physical barrier to its ingestion. This does not seem to be the case in *P. vernalis* Taylor & Jokinen, where the sand grains can compose up to 15% of the diet (Kesler et al., 1986), or in *P. venustula* from the Napostá Grande stream (19%).

Blue-green and filamentous green algae are not important items in the diet of *Biomphalaria*, *Chilina*, and *Physa* in the studied area, as revealed by the low proportion of these items in their crops throughout the year. Other pulmonate species seem to prefer filamentous green algae (Lodge, 1986). Madsen (1992) determined that *Biomphalaria pfeifferi*, *Bulinus truncatus*, and *Lymnaea natalensis* are able to select against blue-green algae. The toxicity of some blue-green algae and their mucopolysac-

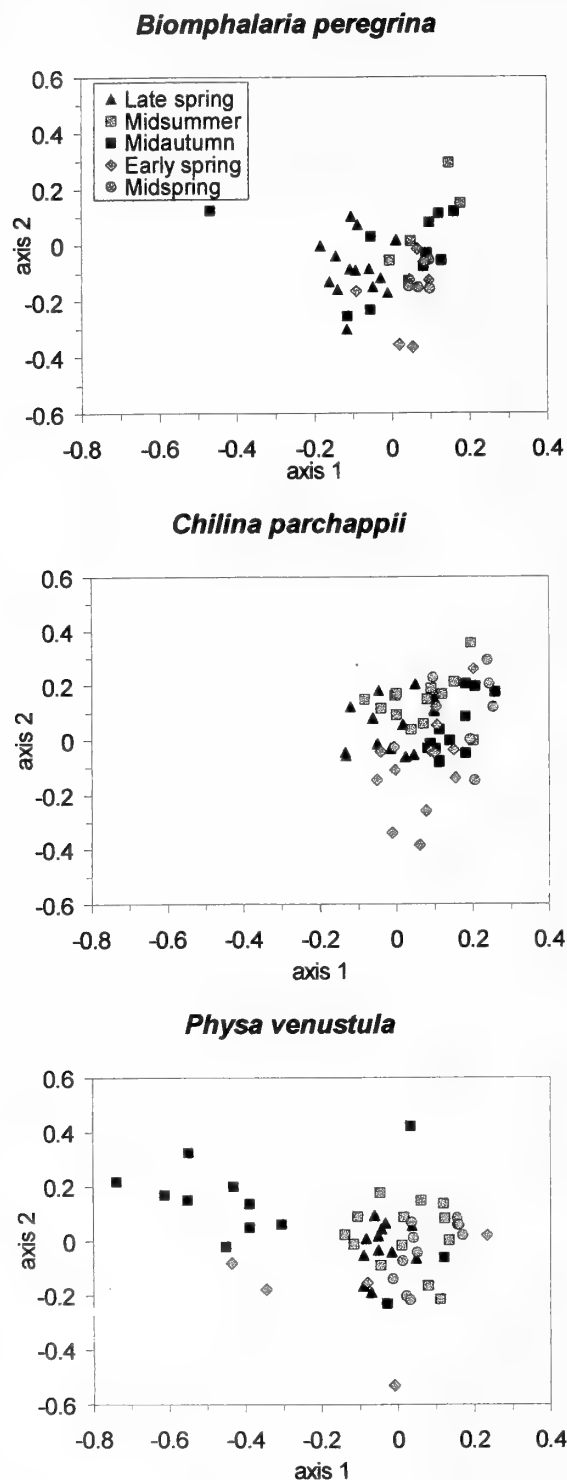


Figure 3. Crop content variations of *Biomphalaria peregrina*, *Physa venustula*, and *Chilina parchappii* based on the first and second principal component scores.

Table 1

Eigenvalues and component loadings for the first two principal components for *Chilina parchappii*, *Biomphalaria peregrina*, and *Physa venustula* based on crop contents.

	PC1 loadings	PC2 loadings
Diatoms (Di)	0.6332	0.7093
Non-filamentous algae (NF)	-0.9230	0.3015
Filamentous green algae (F)	0.0758	0.1232
Blue-green algae (BG)	0.0519	0.1865
Detritus (De)	0.3187	-0.8278
Eigenvalues	0.0297	0.0232
%variance explained	48.82	38.16
F-Value*	14.39	7.16
P	0.00001	0.0011

\* From ANOVA of the three species means of the principal component scores.

charide sheet should account for their low palatability. Some pulmonate species, however, have been successfully reared under laboratory conditions on a diet of blue-green algae (Skoog, 1978; Itagaki, 1987). It is probable that the low concentration of these algae in the stomachs of all of our snails was due simply to their low density in the substrate.

Diatoms eaten by *Biomphalaria*, *Chilina*, and *Physa* showed a variety of forms, sizes, and habits. Underwood & Thomas (1990) pointed out that certain anatomic traits or growing forms of algae can reduce the probability of being swallowed by different species of invertebrates. Hunter (1980) stated that *Cocconeis* is able to escape snail predation as an outcome of its morphology. Nevertheless, Smith (1989) and Dillon & Davis (1991) proposed that snails sample the diatom flora almost randomly, with only a few larger species under-represented in the gut contents. In this study we recorded a diversity of diatoms, from the small ovoid *Cocconeis*, to the elongated and narrow forms of *Synedra*, or the robust *Amphora*. There exists, however, a dominance of mobile diatoms (*Navicula*, *Nitzschia*, *Fragilaria*) and those living attached to the substrate by mucilaginous peduncles (*Cymbella*, *Rhoicosphenia*, *Gomphonema*). Due to their low level of adherence, these diatoms appear to be more vulnerable to attack by snails.

*Biomphalaria peregrina*, *Chilina parchappii*, and *Physa venustula* do not eat macrophytes. The scarcity of macrophyte fragments in their diet is consistent with other reports in the literature (Brönmark, 1990; Underwood & Thomas, 1990; Madsen, 1992). Leaf hardness seems to be one of the main reasons why pulmonates refuse to eat aquatic plants. The loss of a significant number of radular teeth has been correlated with the consumption of macrophyte material, and the use of macrophytes as food has

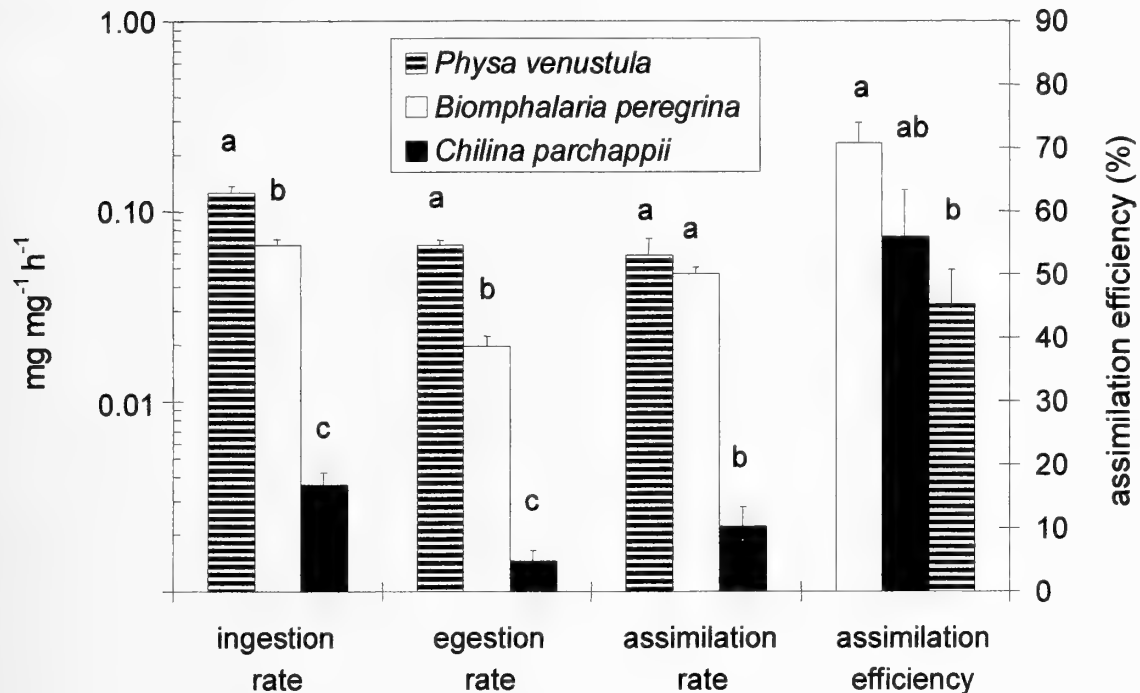


Figure 4. Ingestion rate, egestion rate, assimilation rate, and assimilation efficiencies (mean + SE) of *Biomphalaria peregrina*, *Physa venustula*, and *Chilina parchappii* fed on artificial substrates. Columns sharing the same lower-case letter are not significantly different from each other.

been thought to be energetically disadvantageous (Cedeño-León & Thomas, 1982; Thomas, 1982). Mackenstedt & Markel (1987) determined that the replacement of teeth in some freshwater gastropods is a continuous process and that *Lymnaea*, for example, replaces a whole radula in 24 days. Although aquatic plants do not constitute a food item for *Chilina*, almost half of the specimens swallowed their own radular teeth. The presence of teeth in the stomachs of *Chilina* corresponds therefore to this natural replacement process, and is not a consequence of food hardness.

Ingestion rates calculated for *Chilina parchappii* were much lower than the rates for the other two species. Since the algae colonizing the slides were those normally eaten in the same proportion as they appear on the substrate, this difference was probably due to ethological factors.

The assimilation values found in this study fit within the range already published for other pulmonate species (reviewed by Brendelberger, 1997). The strength of the crop differs from the slender crop of *Physa* to the strong, muscular one of *Chilina*, possibly accounting for observed differences in efficiency rates. *Biomphalaria* and *Chilina* better fit the primitive model of stomach-grinders (Brace, 1983). The highest assimilation efficiency was shown by *Biomphalaria*, which is the species with the highest proportion of sand in the crop. *Physa*, the species with the highest ingestion rate, has the weakest stomach and the lowest efficiency. *Chilina*, with its strong grinding

stomach, compensates for its low ingestion rate and reaches a high assimilation efficiency.

*Chilina parchappii*, endemic and less active than the other species, is subject to a probable risk of competition in a case of food shortage. The potential for food limitation in lotic ecosystems is small, but there is evidence (mostly indirect) suggesting that inadequate food supplies can limit some stream invertebrate populations (Crowl & Schnell, 1990; Hill, 1992).

Competition among snails is highly improbable in the present area of sympatry, i.e., the lower basin of the Napostá Grande stream, which is rich in detritus, but it may occur if the invader *Physa* reaches the oligosaprobic sector of the stream where *Chilina parchappii* is the dominant species. In recent years, *Physa* has advanced some 50 km upstream (Martín, 1999).

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## NOTES, INFORMATION & NEWS

### Designation of a Lectotype for *Succinea grosvenorii* Lea (Mollusca: Gastropoda: Pulmonata)

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The description of *Succinea grosvenorii* Isaac Lea, 1864 (p. 109) consisted of a short diagnosis in Latin and a listing of two collection localities and collectors involved.<sup>1</sup>

In the many species described by Lea, the indication “*Hab.*” (Habitat) has traditionally been considered as defining a type locality for the species. However, in the case of *Succinea grosvenorii*, two localities were listed (1864: 109): “*Hab.*—Santa Rita Valley, Kansas? Mr. H. C. Grosvenor; and Alexandria, Louisiana, J. Hale, M.D.” An objective of this paper is to establish a single type locality for this species. Lea (1867:135) noted “From the two habitats I have some twenty specimens.” He described those from Santa Rita Valley, Kansas, as being “all dead shells and opaque white from partial decomposition.” In contrast, those from Alexandria, Louisiana, he depicted as shells “in a perfect state” and of a “fine bright straw color.” Both of these lots are in collections of the National Museum of Natural History. Dr. Robert Hershler, of the museum, reports that the seven whitish specimens from Santa Rita Valley (USNM 121065) are likely subfossil. The 13 specimens from Alexandria, Louisiana (USNM 117878) still retain the straw color attributed to them by Lea. These latter specimens have been separated into two lots, with 12 shells in one box and a single one in another box. Before discussing labels found in these boxes, I summarize some notes concerning the Lea Col-

lection of the National Museum, these also provided by Dr. Hershler.

The National Museum did not acquire the large Isaac Lea Collection until after Lea’s death in 1886. These were mainly in the form of syntype lots. At the time of World War II, for safekeeping, attempts were made to separate out from syntype lots those specimens thought to have been illustrated by Lea in his various publications. These were referred to as “figured holotypes,” seemingly assuming that Lea had intended them as such. There exists such a “figured holotype” in the case of *S. grosvenorii*.

Of the two boxes with shells of *S. grosvenorii*, noted above, one box contains 12 specimens that were referred to as paratypes, and the other box contains a single specimen, slightly larger than any of the “paratypes,” and which is labeled as a figured holotype. There is a single label in the box of “paratypes” and two labels in the other box with the single specimen. For convenience, these labels are numbered 1–3, below.

Label 1. This label is in the box with 12 shells. It is written on a museum label preprinted with “U. S. Nat. Mus.” and “Lea Coll.” Handwritten is:

117878      *Succinea grosvenori* Lea.  
PARATYPES      Alexandria, La.      Hale.

Perhaps this label was written at the time, in the 1940s, noted above, when “types” were separated for safekeeping.

Label 2. This is one of two labels in the box with a single specimen. This is also a USNM label and with the designations “U. S. Nat. Mus.” and “Isaac Lea Coll.” The words “FIG’D HOLOTYPE” appear in printed handwriting. The other words are in an elegant, cursive style, with flourishes embellishing capital letters, a style still common at least until the latest 1800s.

117878      *Succinea Grosvenori*, Lea  
FIG’D. HOLOTYPE.  
Alexandria La.      Hale

As this is a National Museum label, it must postdate the time of Lea’s death in 1886, after which Lea’s collection was acquired by the museum. Perhaps it is even later than 1906, when Pilsbry, at least, had started to use the spelling *grosvenori*. However, the marked difference in styles of handwriting would suggest that the part of the label in cursive hand was written earlier than the printed words “FIG’D HOLOTYPE,” which might have been inserted on an empty line in the 1940s, as discussed above.

Label 3. This label is in the same box as Label 2. The paper is yellowed with age, and the right side of the label

<sup>1</sup> The patronymic specific name (for H. C. Grosvenor) terminated in *-ii*, the spelling also utilized by Lea (1867:135) in an amplified discussion of this species. Subsequently, the *-ii* ending was used by various other authors including Pilsbry’s catalogue of land snails (1898:143), and even later, as in Shimek (1935). However, Pilsbry & Ferriss used the single *-i* as early as 1906, as did Pilsbry also in his monograph of North American land mollusks (1948:819). In the synonymy of the species in the monograph, he erroneously indicated that the *-i* spelling had been employed in Lea’s original description. Subsequent authors have followed Pilsbry’s example (1948), and employed the single *-i* ending, as in Turgeon, ed. (1998:146). Article 33.4 of the fourth edition of the *International Code of Zoological Nomenclature* (2000:43) indicates that the use of an *-i* ending for a species name originally and correctly employing the *-ii* ending is incorrect. Thus, in this species it seems clear that the correct specific name is *grosvenorii*.

seems to have been torn off, rather than cut. This is not a preprinted USNM label, but is simply blank paper with handwriting, of which the ink is greatly faded. Some words, indicated by (?), are only tentatively identified below. Except for a USNM number written at the bottom of the label in printed handwriting, the remainder is in a cursive style, but less elegant than that exhibited in Label 2.

April 64      *Succinea grosvenorii* Lea  
108      Near to *S. luteola* Gould but differs so (?)  
            probably (?) new  
[“*ovalis* Say” is written above “*luteola* Gould,” per-  
            haps meant as an insert]  
q. v. *campestris* Say  
Dr. Hale      Alexandria, Louisi [end of word torn off]  
USNM 117878 [in printed handwriting, more modern-  
            appearing than that above]

In regard to label 3, April 1864, is the month of publication of the original description of *S. grosvenorii* by Lea, as noted above. The number given to the figure of the species in Lea's *Observations* . . . series (1867: pl. 24) is 108. The presence of this number on the label suggests that it was meant to designate a single specimen rather than the entire lot of syntypes. One might also suspect that Lea himself separated out the specimen and wrote the label. The terse allusions made to related species favor this view, although it is also possible that these remarks were written later at the National Museum by a curator with an interest in succineids.

Regardless of authorship and history of the above labels, it is surely clear that the “straw-colored” syntypes, including the “FIG'D HOLOTYPE,” are from Alexandria, Louisiana. Obviously they are not from “Santa Rita Valley, Kansas.” This locality was followed by a question mark even in Lea's original description. In a search by Ms. Grace Muilenburg, a specialist on the history of Kansas, no place-name incorporating the name Santa Rita was found within the present or former confines of that state. The place-name does occur in New Mexico and is common in northern México.

In the absence of a formally defined holotype for *Succinea grosvenorii*, it seems appropriate to designate the single shell indicated by labels 2 and 3 above as a lectotype. This is done hereby. This specimen (Figure 1) is still in excellent condition and seems likely to be the one that was chosen by Lea as an exemplar meriting illustration. It has almost the same dimensions as the illustration itself in figure 108 in Lea (1867: pl. 24). As the largest of the 13 syntypes, it seems highly likely that Lea gave it this special recognition, as has at least one curator since then, and it seems fitting to continue that “tradition.” As per ICZN Recommendation 76A.2, this action has the desirable effect of formally establishing the type locality of *Succinea grosvenorii* at Alexandria, Louisiana, as has been suggested informally by Hubricht (1963:135). This action also invalidates the enigmatic Santa Rita Valley

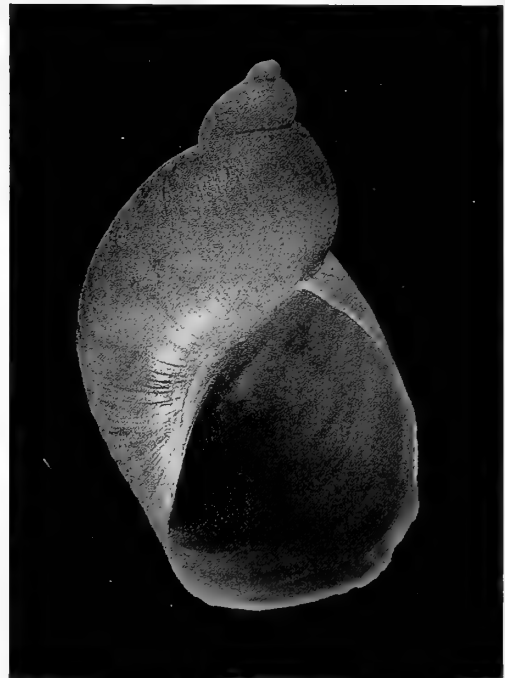


Figure 1. Lectotype of *Succinea grosvenorii* Lea, 1864. United States National Museum number is 117878. Some dimensions (in mm) of the shell are: height, 13.0; width, 7.9; height of aperture, 8.3; and width of aperture, 5.8.

locality as a contender for type locality. The USNM number for the lectotype remains 117878. The lot of 12 paralectotypes (indicated by Label 1, above), all of which are judged to be of the same species as the lectotype, is renumbered USNM 880662.

Height of the lectotype is 13.0 mm. Other dimensions are indicated in Figure 1. The 12 paralectotypes range in height from 9.2 to 12.5 mm with a mean of 11.2 mm.

I thank Dr. Robert Hershler, National Museum of Natural History, for the loan of specimens and for providing the photograph in Figure 1, and I thank both him and Dr. Shi-Kuei Wu, University of Colorado, for useful suggestions. I am grateful to Dr. Elizabeth Walsh and Ms. Laura Dader, University of Texas at El Paso, for assistance in making prints.

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### International Commission on Zoological Nomenclature

The following Applications concerning mollusks were published on 28 September 2001 in Volume 58, Part 3 of the *Bulletin of Zoological Nomenclature*. Comment or advice on any of these applications is invited for publication in the *Bulletin* and should be sent to the Executive Secretary, I.C.Z.N., % The Natural History Museum, Cromwell Road, London SW7 5BD, U.K. (e-mail: iczn@nhm.ac.uk).

Case 2983. *Achatinellastrum* Pfeiffer, 1854 and ACHATINELLIDAE Gulick, 1873 (Mollusca, Gastropoda): proposed conservation.

Case 3192. BULIMINIDAE Kobelt, 1880 (Mollusca, Gastropoda): proposed emendation of spelling to BULIMINUSIDAE, so removing the homonymy with BULIMINIDAE Jones, 1875 (Rhizopoda, Foraminifera); and ENIDAE Woodward, 1903 (1880) (Gastropoda):

proposed precedence over BULIMINUSIDAE Kobelt, 1880.

Case 3153. HIPPOPODIIDAE Cox, 1969 (Mollusca, Bivalvia): proposed emendation of spelling to HIPPOPODIUMIDAE, so removing the homonymy with HIPPOPODIIDAE Kolliker, 1853 (Cnidaria, Hydrozoa).

The following Opinion concerning mollusks was published on 29 June 2001 in Volume 58, Part 2 of the *Bulletin of Zoological Nomenclature*. Copies of Opinions can be obtained free of charge from the Executive Secretary, I.C.Z.N., % The Natural History Museum, Cromwell Road, London SW7 5BD, U.K. (e-mail: iczn@nhm.ac.uk).

Opinion 1973 (Case 3126). *Bulinus wrighti* Mandahl-Barth, 1965 (Mollusca, Gastropoda): specific name conserved.

The following Opinions concerning mollusks were published on 28 September 2001 in Volume 58, Part 3 of the *Bulletin of Zoological Nomenclature*.

Opinion 1979 (Case 3086). *Hyalinia villae adamii* Westerlund, 1886 (currently *Oxychilus adamii*; Mollusca, Gastropoda): specific name *adamii* conserved by the replacement of the syntypes with a neotype.

Opinion 1980 (Case 3088). *Doris verrucosa* Linnaeus, 1758 (Mollusca, Gastropoda): generic and specific names conserved by the designation of a neotype.

Opinion 1981 (Case 3133). *Peristernia* Mörch, 1852 and *Clivipollia* Iredale, 1929 (Mollusca, Gastropoda): conserved by the designation of *Turbinella nassatula* Lamarck, 1822 as the type species of *Peristernia*.



## BOOKS, PERIODICALS & PAMPHLETS

### **Evolutionary Biology of the Bivalvia**

edited by E. M. HARPER, J. D. TAYLOR & J. A. CRAME.  
2000. Geological Society Special Publication No. 177.  
The Geological Society, Burlington House, Piccadilly,  
London, United Kingdom W1V 0JU. 494 pp.

*Evolutionary Biology of the Bivalvia* represents the most important volume on this topic to be published in the 20 years since “Evolutionary systematics of bivalve molluscs” (1978. Philosophical Transactions of the Royal Society of London, B. pp. 199–436). The text follows closely on the heels of Johnston & Haggart (1998), and together these volumes summarize the current state of our knowledge of the evolution of the Bivalvia. However, whereas the Johnston & Haggart volume (1998) had the distinct feeling of a volume compiled after a 20 year hiatus (albeit an important compilation), the current text is more definitive in its impact.

The opening papers by Steiner & Hammer (“Molecular phylogeny of the Bivalvia inferred from 18S rDNA sequences with particular reference to the Pteriomorpha”) and Campbell (“Molecular evidence on the evolution of the Bivalvia”) report on molecular-based cladistic analyses (18S rDNA) of overall bivalve phylogeny, the divergence of major divisions such as the Pteriomorpha and Heteroconchia, and the possible evolutionary roots of the Bivalvia. These reports are an important step forward in addressing bivalve phylogeny at all levels, and can only be expected to improve as the number of sequenced taxa increases. Both studies also complement well previous morphology-based analyses, such as the outstanding report by Waller (1998). Waller’s conclusions, based on detailed observational analyses of morphological (including conchological) and developmental characters, are corroborated significantly at higher taxonomic levels by the molecular analyses. This highlights the indisputable value of molecular analyses; they utilize data that are independent of morphological characters (in an analytical, not biological sense), and therefore “are less selected by lifestyle and habitat than is morphology” (Steiner & Hammer). Nevertheless, our reconstruction of bivalve phylogenies cannot rely upon molecules alone, since that would ignore the wealth of understanding of the developmental and functional bases of shell morphology, a long tradition of soft-anatomical work, and a fossil record that is one of the best for any metazoan taxon.

This point is emphasized by many of the remaining phylogenetic papers in the volume. There are higher level phylogenetic reconstructions based on morphological data

(Carter et al.), as well as detailed and current investigations of the phylogenetic importance and origin of early Paleozoic taxa (Cope). Bivalvia is one of only a handful of taxa with Cambrian and Ordovician records rich enough to permit such reconstructions. The work is hampered, however, by factors such as character interpretations, time-dependent convergence, and preservation. Increased gene sequencing will ultimately circumvent many of these problems, but morphological and paleontological studies must continue; in one sense, molecular analyses are both easier and evolutionarily less illuminating. We have solid theoretical models of sequence evolution upon which to base parsimony and maximum likelihood analyses, though we lack the information necessary to understand most of the phenotypic implications of that evolution. Morphology-based analyses unfortunately lack the models of character state transformation that are at the heart of gene sequence-based analyses. Hopefully, this situation will change with the eventual work on developmental genetics and gene function in bivalve taxa. Until that time, however, morphological interpretations must continue to rely upon ecological, functional morphological, developmental, and morphometric analyses.

Fortunately, these all find impressive representation in the volume. Following the phylogenetic analytical papers are a series of contributions examining the evolutionary biology of a variety of bivalve characters. These characters range from cell and tissue-level examinations of sperm, sensory structure, gastric and respiratory structures, to theoretical and empirical functional examinations of shell structure and mechanics. While most of these studies are at the rank of family or above, there are a number of papers dealing with generic and species-level taxa. The focus here is primarily on examinations of genetic and phenotypic variation, or the environmental bases of such variation; these topics have been traditionally well represented in bivalve research, and the tradition continues in the present volume with the application of new tools, such as nuclear DNA variation and modern morphometric analysis.

Finally, analyses of biogeographic patterns and regional biodiversity by Crame, Jablonski et al., and Mikkelesen & Bieler emphasize the broad applicability of bivalve biology. This point in fact highlights the dual nature of this volume. This text presents many of the most current ideas explaining the evolution of the Bivalvia at multiple levels. While these studies advance our knowledge of this important taxon, they also serve notice that major contributions to our understanding of animal evolution, evolu-

tionary ecology, biogeography, and paleobiology/paleoecology will continue to be made by bivalve workers. In the end, however, the reader is left with the distinct and correct impression that our understanding of these topics as they apply to the Bivalvia (and vice versa) is still very incomplete. And that, perhaps, will be the most significant contribution of this volume to the next 20 years of research.

**Peter D. Roopnarine**

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#### **Tropical Deep-Sea Benthos, Volume 22**

edited by PHILIPPE BOUCHET & BRUCE A. MARSHALL. 2001. *Mémoires du Muséum National d'Histoire Naturelle* 185:1–407, 638 figures, 4 color plates. ISBN 2-85653-527-5.

The series *Tropical Deep-Sea Benthos*, a continuation of *Résultats des Campagnes MUSORSTOM*, is dedicated to inventorying and describing the deep ocean faunas of the world, with special emphasis on the Indo-Pacific realm. The present volume is the fourth "all-mollusk" volume in the series (previous ones being: 7 [1991], 14 [1995], and 19 [1998]). It contains contributions by ex-

perts from Australia, New Zealand, Belgium, France, the Netherlands, Russia, Taiwan, and the United States, and includes papers on Aplacophora, Polyplacophora, Bivalvia, Gastropoda, and Cephalopoda.

The contributions are largely based on material collected by a series of dredging and trawling expeditions conducted since 1985 in the south Pacific under the leadership of Dr. Bertrand Richer de Forges, using the Nouméa-based Research Vessel *Alis*. Earlier expeditions covered New Caledonia, Vanuatu, Fiji, Wallis and Futuna, Tonga, and the Marquesas. Plans for the coming years include the Solomon and Austral Islands. Series editor Philippe Bouchet (in litt.) estimates that the resulting south Pacific deep-sea collections contain on the order of 2000 new molluscan species; it will take many more years and the input of malacologists from all over the world to document and appropriately describe this fauna.

Contents of volume 22 include descriptive systematic papers on Prochaetodermatidae (Aplacophora) by D. L. Ivanov and A. H. Scheltema; Polyplacophora by B. Sirenko; bathyal Pectinoidea (Bivalvia) by H. H. Dijkstra; the limid genus *Acesta* (Bivalvia) by B. A. Marshall; Spondylidae (Bivalvia) by K. L. Lamprell and J. M. Healy; Poromyidae (Bivalvia) by E. M. Krylova; Triviidae (Gastropoda) by L. Dolin; Muricidae (Gastropoda) by R. Houart; turritiform gastropods by A. Sysoev and P. Bouchet; deep-water Pleurobranchaeidae (Gastropoda) by B. Dayrat; phylidiid nudibranchs (Gastropoda) by A. Valdés; and cephalopods from waters around Wallis and Futuna by C.-C. Lu and R. Boucher-Rodoni.

Other volumes in *Tropical Deep-Sea Benthos* deal with other groups of marine invertebrates and fishes. An overview can be seen on the website of the Muséum National d'Histoire Naturelle, [www.mnhn.fr/publication/memoire/mem.html](http://www.mnhn.fr/publication/memoire/mem.html).



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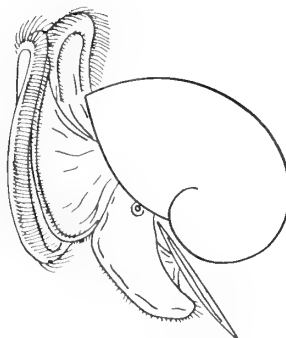
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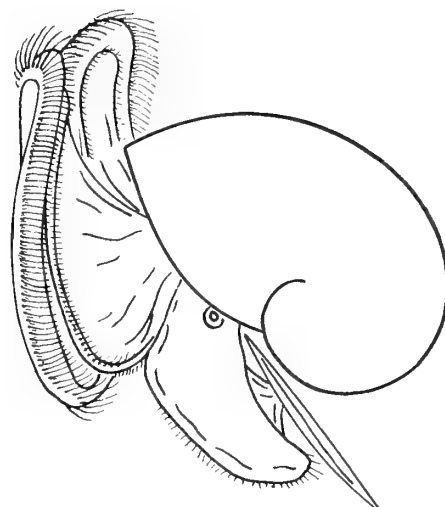


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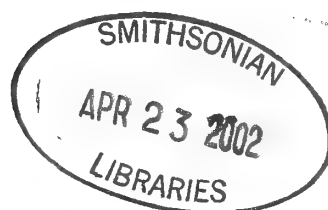
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## THE VELIGER

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# The Genus *Nodilittorina* von Martens, 1897 (Gastropoda: Littorinidae) in the Eastern Pacific Ocean, with a Discussion of Biogeographic Provinces of the Rocky-Shore Fauna

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**Abstract.** The Recent members of the genus *Nodilittorina* in the eastern Pacific Ocean are revised. Hitherto eight to 10 species have been recognized, but this total is now increased to 18, of which three are named as new. The majority of the taxa fall into three species complexes: six in the *N. porcata* group, two in the *N. modesta* group, and six in the *N. aspera* group. Within each of these complexes, species identification from shells alone is difficult, as a result of remarkable intraspecific variation. Since all species of the genus have pelagic eggs and planktotrophic development, it is suggested that this variation may be partly of ecophenotypic origin. Discrimination is confirmed by species-specific characters of the penis and supported by some features of the spermatozoa and pallial oviducts. Radular characters are more constant throughout the genus. The four additional species are *N. araucana*, *N. peruviana*, *N. galapagensis*, and *N. fernandezensis*. Anatomical features, radulae, and a range of shells are figured for each species. Geographical distributions are mapped in detail (from 777 samples examined) and cases of sympatric occurrence provide strong support for the discrimination of members of the three species complexes.

There is insufficient morphological differentiation among the species to permit formal phylogenetic analysis. Some of them show similarities with congeners in the western Atlantic, but there are no obvious sister-species pairs. A species endemic to the oceanic islands off Chile, *N. fernandezensis*, shows a clear relationship with a largely temperate Southern Hemisphere group, the subgenus *Austrolittorina* (here redefined).

Of particular interest are the distributions of the 15 *Nodilittorina* species within the Tropical Eastern Pacific Region (TEP; hitherto referred to as the “Panamic Province” in the molluscan literature). These strongly support the classification of the region into four provinces, Cortez, Mexican, Panamic (with a southern Ecuadorean element), and Galápagos, as previously suggested for fishes, which (like *Nodilittorina* species) are dependent upon shallow-water rocky substrates. The boundaries between these provinces correspond with habitat gaps, of either open water (Galápagos) or inhospitable coastline of sand, mud, and mangroves (Sinaloa, Central American and Colombian Gaps). The implications for processes of dispersal and speciation, and also for future systematic studies of the rocky-shore fauna, are discussed.

## INTRODUCTION

The eastern Pacific Ocean stretches from the shores of Alaska to Tierra del Fuego, and includes tropical islands as far west as the Islas Revillagigedo, Clipperton Atoll, the Galápagos, and the Juan Fernández Archipelago. Within this expanse there occur three principal genera of the littorinid subfamily Littorininae (using the classification of Reid, 1989a). The northern temperate genus *Littorina* includes seven native species in this region, of which two extend as far south as Baja California (Reid, 1996). The genus *Littoraria* is exclusively tropical and includes five species from mangrove habitats and one from rocky shores (Reid, 1999a). The remaining members of the subfamily that are of native occurrence and maintain reproducing populations are all members of the genus *Nodilittorina*; these can be found from Baja California to southern Chile and on all the oceanic islands. In addition to these eastern Pacific natives, there are three introduced *Littorina* species recorded from San Francisco Bay and

Washington (Reid, 1996), and *Cenchritis muricatus* (L.) has been introduced to the Gulf of California (Bishop, 1992; Chaney, 1992). Recently, three tropical species of Indo-West Pacific origin have been recorded from the eastern Pacific for the first time, two of *Littoraria* and one of *Peasiella*, but these appear to be only occasional immigrants (Reid & Kaiser, 2001).

*Nodilittorina* is the largest genus in the family Littorinidae, with an estimated 60 species worldwide. Most of these are tropical, and in the temperate northern hemisphere they are replaced (with little geographical overlap) by species of *Littorina*. In contrast, in the southern hemisphere, *Nodilittorina* species extend throughout the temperate latitudes. All the species occur typically on intertidal rocks, usually in the littoral fringe and upper eulittoral zone, where they graze on epilithic and endolithic algae. They are usually the dominant large invertebrates at these levels on the shore, and have therefore been the subject of much work on ecology and physiology. Ex-

amples include studies in Australia (Underwood & McFadyen, 1983; Chapman, 1994, 1998), South Africa (McQuaid, 1981a, b, 1992), Hong Kong (Ohgaki, 1985; Mak, 1998; Mak & Williams, 1999), Japan (Ohgaki, 1988, 1989), Hawaii (Struhsaker, 1968), the Mediterranean (Palant & Fishelson, 1968), Brazil (de Magalhães, 1998), and the Caribbean (Borkowski, 1974; Britton, 1992; Lang et al., 1998; see also McQuaid, 1996b, for a review of littorinid ecology). The ecology of these littorinids has, however, received relatively little attention in the eastern Pacific region (Vegas, 1963; Markel, 1971; Vermeij, 1973; Jordan & Ramorino, 1975; Garrity & Levings, 1981; Garrity, 1984), although they have often been mentioned in studies of littoral zonation (Hedgpeth, 1969; Cinelli & Colantoni, 1974; Paredes, 1974; Romo & Alveal, 1977; Santelices et al., 1977; Brattström, 1990).

Despite this ecological importance, the taxonomy of *Nodilittorina* species remains poorly understood. As discussed below, even a satisfactory definition of the genus is not available. There have been no modern studies of the systematics of this group in the eastern Pacific. The first species to reach European collections was the Peruvian *N. peruviana*, named by Lamarck (1822), and on his great South American journey, d'Orbigny (1835–1846) obtained both this and the Chilean *N. araucana*. Further material was brought back by Cuming, from which Philippi (1846a) described three tropical species. There soon followed taxonomic studies on material from Mexico (Menke, 1851; Carpenter, 1857b, 1864a), Central America (C. B. Adams, 1852a, b; Mörch, 1860; Carpenter, 1863; von Martens, 1900), and the Galápagos (Stearns, 1892, 1893a, b). Meanwhile, monographs of worldwide littorinids had appeared in the great nineteenth century conchological iconographies (Philippi, 1846b–1848; Küster, 1853, 1856; Reeve, 1857–1858; Weinkauff, 1878, 1882; Tryon, 1887). With limited material available, the earlier authors distinguished many shell variants as species; for example, Philippi (1847) included eight in this eastern Pacific group. Later, the trend was to synonymize many names; Weinkauff (1883) accepted only six of Philippi's taxa, Tryon (1887) only four, and Dall (1909) likewise had a broad concept of species in this group. Twenty-eight names were introduced in the nineteenth century, and three more in the twentieth (Bartsch & Rehder, 1939; McLean, 1970; Rosewater, 1970). For much of the twentieth century, authors continued to accept a wide degree of intraspecific variation in species that were defined by shell characters alone, so that the influential *Sea Shells of Tropical West America* by Keen (1971, following taxonomy of Rosewater, 1970) included only eight (plus seven additional names listed uncritically as Fossaridae, see discussion of *N. porcata* group). This book has been the basis for several regional faunal lists (Finet, 1985, 1994; Alamo & Valdivieso, 1987, 1997; Kaiser, 1997). The modern taxonomy of the Littorinidae has been transformed by the use of anatomical and radular characters,

correlated with details of shell sculpture and pattern (e.g., Bandel & Kadolsky, 1982; Reid, 1986, 1989a, 1996), and this has led to a proliferation of recognized species and to more rigorous phylogenetic definitions of genera. In a list of worldwide Littorinidae Reid (1989a) gave eight species in this eastern Pacific group (with two additional species doubtfully in synonymy) and, for the first time, all were included together in *Nodilittorina*.

Many workers have commented on the confusing variability and uncertain taxonomy of the littorinids of the eastern Pacific, especially those related to *Nodilittorina porcata* and *N. aspera*. In 1971 Keen remarked of "*Littorina aspera*" that "it is possible that careful work will demonstrate the desirability of recognizing more than one species within this complex." However, despite advances in littorinid taxonomy in other parts of the world, the eastern Pacific *Nodilittorina* species have remained neglected. The present study aims to revise the taxonomy of this group, based on personal field collections and examination of the major museum collections. Particular emphasis is placed on characters of the reproductive system (penis, paraspermatozoa, pallial oviduct, egg capsules) which are known to be important for the discrimination of littorinid species (Reid, 1986, 1996, 1999a). Fossil material has not been included. In general, fossil littorinids are extremely scarce, as expected for a group living primarily on hard intertidal substrates. Furthermore, the shells of *Nodilittorina* species are so variable and also show such close resemblance to some members of *Littoraria* and *Littorina*, that fossil material would be difficult to interpret. The only possible fossil member of the genus *Nodilittorina* that has been recorded from tropical America is *Littorina seminole* Petuch from the Pliocene Caloosahatchee Formation of Florida (Petuch, 1991).

As a result, 18 *Nodilittorina* species are recognized in the eastern Pacific Ocean, where hitherto only eight to 10 were generally accepted. Their geographical distributions are plotted in detail, and congruent patterns among species complexes, as well as cases of sympatry, provide support for the new species definitions. Of particular interest are the distributions of the 15 species within the Tropical Eastern Pacific Region (TEP) which suggest its division into four provinces, Cortez, Mexican, Panamic, and Galápagos. Although this division has previously been recognized in some other animal groups, in the molluscan literature the entire TEP has been regarded as uniform and referred to as the "Panamic Province." The boundaries between these provinces correspond with habitat gaps, of either open water or inhospitable coastline of sand, mud, and mangroves. The recognition of these gaps has important implications for systematic, evolutionary, and genetic studies of the rocky-shore fauna.

## MATERIALS AND METHODS

During this study, all material in the collections of the following institutions has been examined: the Natural

History Museum, London (BMNH), the National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM), the Muséum National d'Histoire Naturelle, Paris (MNHN), and the Zoölogisch Museum, Amsterdam (ZMA). Much additional material, of species and from geographical regions that were otherwise poorly represented, has been obtained from the Los Angeles County Museum of Natural History (LACM), the California Academy of Sciences (CAS), the Charles Darwin Research Station, Ecuador (CDRS) and the personal collections of K. L. Kaiser (KLK) and G. J. Vermeij. All available primary type material has been examined (in one case a photograph) from these institutions, and also from the Academy of Natural Sciences of Philadelphia (ANSP), the Museum of Comparative Zoology, Harvard University (MCZ), the Muséum d'Histoire Naturelle, Geneva (MHNG), the Museum für Naturkunde, Berlin (MNB), and the Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt (SMF). Paratypes of some species are housed in the American Museum of Natural History (AMNH) and in the Santa Barbara Museum (SBM), but these were not examined. Personal collections were made in Costa Rica (1985), Mexico (1994), Chile and Peru (1998), and Ecuador (1998), and are deposited in BMNH. In total, 777 samples have been examined. Fossil material is extremely scarce and has not been included in this study.

Shell dimensions were measured with vernier calipers or, for the smallest species, with a camera lucida and scale, to 0.1 mm. Shell height (H) is the maximum dimension parallel to the axis of coiling, shell breadth (B) the maximum dimension perpendicular to H, and the length of the aperture (LA) the greatest length from the junction of the outer lip with the penultimate whorl to the anterior lip. Shell shape was quantified as the ratios H/B and H/LA (relative spire height, SH), and the range of these ratios is quoted. Shell sculpture is described by reference to primary and secondary grooves, ribs, the periphery, and microstriae. Primary grooves are incised spiral lines or grooves that are visible on the early teleoconch whorls. On the spire the primary grooves are counted between successive sutures, but on the last whorl they are counted from the suture to the periphery of the whorl (so that one or more extra grooves are visible). Secondary grooves appear by interpolation, usually on the penultimate or final whorl, and sometimes not at all. The spaces between the grooves are referred to as ribs, whether or not they are strongly raised. Microstriae are fine incised spiral lines that cover the entire surface and are visible only under low magnification; they may be present in addition to primary grooves, but in the smallest species (the *N. porcata* group) the distinction between primary grooves and microstriae is sometimes arbitrary. The periphery is the junction between the upper part of the final whorl and the base of the shell; it is usually marked by a strong or slight angulation (e.g., Figure

18R), or by a rib slightly larger than the rest (e.g., Figure 12B), or more rarely by a keel or flange (e.g., Figure 1N). The suture generally runs one or two ribs above the periphery, or is situated at the peripheral rib. Protoconch whorls were counted as recommended by Reid (1996); the protoconch in Figure 22G has 2.7 whorls. To describe the coiling of the operculum, the opercular ratio was defined as the ratio of two parallel measurements, the diameter of the spiral part divided by the maximum length (Reid, 1996). The relative radular length is the total radular length divided by shell height.

Living animals were relaxed in 7.5% (volume of hydrated crystals to volume of fresh water) magnesium chloride solution. Animals were fixed in 10% seawater formalin buffered with borax, and stored in 80% ethanol before dissection. Anatomical drawings were made by camera lucida, and shading and drawing conventions are indicated in the captions of Figures 3, 4, and 13. For general accounts of the anatomy of littorinids, see Reid (1986, 1989a, 1996). Sperm samples were removed from the seminal vesicles of relaxed, living animals, fixed in 0.5% seawater formalin, examined immediately by light microscopy, and drawn by camera lucida. Alternatively, sperm were removed from specimens fixed and stored in seawater formalin, but not from material stored in ethanol (in which shrinkage of paraspermatozoa by about 20% occurs, Reid, 1996). For four species, egg capsules were obtained by confining individual females in beakers of seawater left overnight; these were drawn using a compound microscope and camera lucida. Radulae were cleaned by soaking in a hypochlorite bleaching solution at room temperature for about 5 min, rinsed in distilled water, mounted on a film of polyvinyl acetate glue on glass, allowed to dry in air, and coated with gold and palladium before examination in a scanning electron microscope. Unworn portions of radulae were viewed in three orientations: in standard flat view from vertically above the radula (to show shapes of tooth bases), at an angle of 45° from the front end of the radula (to show shapes of tooth cusps), and at an angle of 45° from the side of the radula (to show relief). The shape of the rachidian tooth was quantified as the ratio of the total length (in flat view) to the maximum basal width.

Synonymies are not exhaustive, but attempt to list all new names (including nomina nuda) and new combinations, major taxonomic works and faunistic lists, and significant morphological descriptions. Where valid names are represented by syntypic series of dry shells, lectotypes have been designated. This is considered necessary for stability, since identification from shells alone can be difficult (ICZN, 1999, Art. 74.7.3). Lectotypes are also designated in cases where syntypes are not all conspecific.

Distribution maps were plotted from the material examined, with the addition of literature records (where these extended the known range and were considered reliable). Localities are listed only when they are range lim-

its or are of other biogeographic significance. Species are common throughout the range except where noted otherwise. Numbers of specimens in lots are given only for rare and occasional occurrences at the limits of ranges.

### EXCLUDED SPECIES

*Littorina anglostoma* C. B. Adams, 1852

Examination of the lectotype (MCZ 186442) from Panama has shown this to be an *Iselica* (Pyramidellidae). It was figured by Turner (1956:pl. 13, fig. 1).

*Fossarus guayaquilensis* Bartsch, 1928

The original figure (Bartsch, 1928:fig. I, 6) shows that this species from Ecuador is indeed a *Fossarus* (Planaxidae). It should not be confused with the manuscript name "*Lacuna guayaquilensis* Bartsch" (see *Nodilittorina santelena* sp. nov.).

*Littorina (Melarhappe) philippii* var. *latistrigata* von Martens, 1900

Although said to come from western Costa Rica, two unregistered synypes in MNB belong to the Caribbean species *Nodilittorina interrupta* (C. B. Adams, in Philippi, 1847).

*Littorina* (?) *megasoma* C. B. Adams, 1852

As noted by Turner (1956), this Panamanian shell is a *Fossarus*. The holotype (MCZ 186419) was figured by Turner (1956:pl. 11, fig. 6).

*Littorina phasianella* Philippi, 1849

This is a species of *Tricolia* (Turbinidae) (see Keen, 1971) with type locality Panama.

*Littorina umbilicata* d'Orbigny, 1840

Although sometimes listed as a littorinid (see Taxonomic History of *N. atrata*), examination of the type series (BMNH 1854.12.4.366) shows this species from Peru and Chile to be a *Tricolia*, as also observed by Keen (1971).

### SYSTEMATIC DESCRIPTIONS

#### Family LITTORINIDAE Anonymous, 1834

#### Genus *Nodilittorina* von Martens, 1897

*Littorina* (*Nodilittorina*) von Martens, 1897:204 (type by subsequent designation, Abbott, 1954, *Littorina pyramidalis* Quoy & Gaimard, 1833)

*Littorina* (*Echinolittorina*) Habe, 1956:96–99 (type by original designation *Littorina tuberculata* Menke, 1828; cited as *Echinolittorina* in error, p. 96)

*Granulilittorina* Habe & Kosuge, 1966:313–314, 328 (type by monotypy *Granulilittorina philippiana* Habe & Kosuge, 1966 = *N. vidua* (Gould, 1859))

*Littorina* (*Austrolittorina*) Rosewater, 1970:467 (type by original designation *Littorina unifasciata* Gray, 1826)

*Littorina* (*Fossarilittorina*) Rosewater, 1981:29 (type by original designation *Phasianella meleagris* Potiez & Michaud, 1838)

**Taxonomic history:** The recognition and definition of the genus *Nodilittorina* has a long and complex history. In classifying this group, authors have employed characters of the shell, operculum, radula, reproductive anatomy, and egg capsules. Since the groupings suggested by these characters do not coincide, there has been corresponding disagreement about generic classification.

The authors of the early iconographies of littorinids (Philippi, 1846b–1848; Küster, 1853, 1856, continued by Weinkauff, 1878, 1882; Reeve, 1857–1858) used only the single inclusive genus *Littorina* (corresponding to the subfamily Littorininae as currently recognized, Reid, 1989a; intentionally emended to *Littorina* by some nineteenth century authors, see Reid, 1996:39). However, Gray (1839, 1847) had already advocated removal of the large, spinose species with mesospiral or multispiral operculum (corresponding to *Tectarius*; see Reid & Geller, 1997, for history of this genus), while retaining small, nodulose species with typical paucispiral operculum (e.g., *Nodilittorina trochoides* (Gray) and *N. pyramidalis* in current classification) in *Littorina* (Gray, 1839, 1857). This system was modified by Adams & Adams (1854); of the current members of *Nodilittorina*, those with non-descript turbate shells were retained in *Littorina*, those more elongate and brightly patterned were separated as the subgenus *Melarhappe*, whereas those with nodulose sculpture were added to the genus *Tectarius*. A similar division, based on shell and opercular characters, was followed by Tryon (1887). Troschel (1858; followed by Weinkauff, 1883) divided current *Nodilittorina* species between *Littorina* (*Littorina*) and *Littorina* (*Tectus*) (= *Tectarius*) and, although this was based on the supposed narrow rachidian tooth of the latter, and opercular differences, it corresponded once again to a distinction between relatively smooth-shelled and nodulose forms. Shell characters likewise provided a poor guide to the relationships of a neglected group of small, umbilicate shells (here referred to as the *N. porcata* group), variously referred to *Littorina* (Philippi, 1846a, 1847; C. B. Adams, 1852a, b), *Fossar* (Adams & Adams, 1854), *Fossarus* (Carpenter, 1863; Tryon, 1887; Keen, 1971), and several other genera.

The name *Nodilittorina* was introduced by von Martens (1897), as a subgenus of *Littorina*, for those species with nodulose sculpture but a "typical" aperture and operculum (therefore in contrast to *Littorina* (*Tectus*) with a columellar tooth and rounded, many-whorled operculum). Although this new subgenus was accepted in the influential classification of Thiele (1929; followed by Wenz, 1939; Clench & Abbott, 1942), it was placed in *Tectarius* on account of the narrow central tooth, and despite dissimilarity in the operculum. Meanwhile, the use of *Littorina* (*Melarhappe*) for the elongate, patterned species was well established (von Martens, 1897, 1900; Thiele, 1929; Wenz, 1939; Bequaert, 1943). In 1954 Abbott made an important advance by including details of the form of

the penis and of egg capsules in his revision of littorinid genera. On this basis he raised *Nodilittorina* to a full genus and showed clearly that it was more closely related to "*Littorina* (*Melarhaphe*) *ziczac* (Gmelin)" (i.e., the *N. ziczac* group of the western Atlantic) than to *Tectarius*. Abbott (1954) also noted that "*L.*" *ziczac* and "*L. mauritiana*" (a misidentification of *N. unifasciata*) might well not belong to *L. (Melarhaphe)*, depending upon the penial shape of its type species, *M. neritoides* (L.). Shortly afterward, Habe (1956) introduced the subgenus *N. (Echinolittorina)* for *N. tuberculata*, because its rachidian tooth was narrower than that of the type of *Nodilittorina* (*N. pyramidalis*). The new genus *Granulilittorina* was subsequently added by Habe & Kosuge (1966) for a species with unusual serrated egg capsules (*N. vidua*).

In two monographs of Indo-Pacific littorinids, Rosewater (1970, 1972) included a worldwide list of species and presented a revised generic classification. Although characters of penis, egg capsule, and radula were considered, this scheme still emphasized similarities of shell sculpture. Thus the smooth-shelled species currently assigned to *Nodilittorina* were distributed among five subgenera of *Littorina*, the nodulose forms placed in *Nodilittorina* s.s. and its subgenus *Echinolittorina*, and granulose species in *N. (Granulilittorina)*. Rosewater (1970) noted that while nodulose sculpture was "the most obvious character of *Nodilittorina*" it showed considerable variability within some species. Nevertheless, smooth-shelled species with penes closely similar to those of *Nodilittorina* species were placed in a new subgenus *Littorina* (*Austrolittorina*). This scheme was widely followed (e.g., Keen, 1971; Abbott, 1974). Additional information on penes and radulae resulted in transfer of three more members to *Austrolittorina* from the subgenus *Littorina* (Ponder & Rosewater, 1979). A further modification (Rosewater, 1981) was the description of another new subgenus, *Fossarilittorina*, for the small, umbilicate species, but this was still assigned to *Littorina*.

It was not until the work of Bandel & Kadolsky (1982) on western Atlantic *Nodilittorina* species that the genera of Littorinidae were revised to take account of all available evidence. Using published accounts of penes, spawn, and development, and new information on radulae, opercula, and shell mineralogy, they redefined *Nodilittorina* to include, for the first time, both smooth-shelled and nodulose species. They showed not only that shell sculpture was a poor indication of relationships, but also that the rachidian tooth and operculum (both emphasized by earlier authors) were widely variable and subject to evolutionary convergence within *Nodilittorina*. They recognized three subgenera: *Nodilittorina* s.s. (the great majority of species), *Tectininus* (for *N. antonii* (Philippi)), and *Liralittorina* (for *N. striata* (King & Broderip), later removed to *Littorina*, see Reid, 1989a). They also raised *Fossarilittorina* to full generic status. This unfamiliar arrangement was not, at first, widely followed. However,

Reid (1986), in a monograph of *Littoraria*, added additional anatomical data on many *Nodilittorina* species, besides new characters of spermatozoa and pallial oviduct, and presented a preliminary cladistic analysis of the family. This almost entirely supported the new scheme (although *antonii* was removed to *Echininus*).

The most recent revision of the classification of Littorinidae was by Reid (1989a), based on a cladistic analysis of a wide range of morphological characters from examples of all known subgeneric groups. In this new scheme 40 species were placed in *Nodilittorina*, but a precise definition of this genus remained elusive since the only synapomorphy discovered was a dubious character of head pigmentation that was not uniquely derived. Subgenera within *Nodilittorina* were also not clearly defined; three were recognized, *Fossarilittorina*, *Echinolittorina*, and *Nodilittorina* s.s., but lacked strong synapomorphies. In the past decade this generic classification of the Littorinidae has become widely accepted (see reviews by McQuaid, 1996a; Reid, 1996; Reid & Geller, 1997).

**Diagnosis:** Shell: conical to globular; occasionally with pseudo-umbilicus; often an eroded parietal area; adult sculpture of spiral grooves, with or without spiral microstriae, sometimes with granular or nodular sculpture, or sometimes becoming entirely smooth; mineralogy aragonitic, of crossed-lamellar structure with fine outer layer. Head-foot: tentacles pale with 2–3 longitudinal black stripes, or all black. Male: prostate gland open; anterior vas deferens open; penial vas deferens usually open; usually a single mamilliform gland and a penial glandular disc, borne together on a projection of penial base, but either or both may be absent. Paraspermatozoa: usually with rod-pieces. Female: copulatory bursa in relatively posterior or anterior position; egg groove of pallial oviduct coiled in a single spiral of one whorl through albumen gland, sometimes an additional loop in capsule gland and/or in jelly gland. Spawn and development: pelagic capsules, usually cupola-shaped with concentric rings on upper side, containing single ova; development planktonic. Radula: rachidian tooth longer than wide, sometimes considerably narrowed, 3 cusps, central one elongate; 4 cusps on each of lateral and inner marginal (occasionally reduced), but one major cusp on each is elongate; outer marginal with narrowed neck and projection on outer side of base, 4–10 cusps (modified from Reid, 1989a).

**Remarks:** The phylogenetic analysis of Reid (1989a) was based on a morphological survey of a large number of littorinid species, including 35 assigned to *Nodilittorina*, but in the cladistic analysis these were represented by a single example from each of the supposed subgeneric groups, together with a few species of uncertain relationships. The genus *Nodilittorina* was represented by *N. (Fossarilittorina) meleagris*, *N. (? Fossarilittorina) modesta*, *N. (Echinolittorina) dilatata* (d'Orbigny), and *N.*

(*Nodilittorina*) *pyramidalis*. In the resulting cladogram, the genus appeared as a monophyletic group, but the only synapomorphy was coloration of the head, considered to be a weak character since it was shared with two unrelated groups. It appeared therefore that most of the character states used in the diagnosis (see above) were plesiomorphic and not indicative of close relationship. Three subgenera were tentatively distinguished, based on absence of penial glands (*Fossarilittorina*) and position of the copulatory bursa (at the posterior end of the straight section of the pallial oviduct in *Echinolittorina*, anterior in *Nodilittorina* s.s.), but again these character states were not unique or even (in the case of the posterior bursa) apomorphic. There was a suggestion of some correlation with biogeography, since *Fossarilittorina* and *Echinolittorina* were restricted to the Atlantic and eastern Pacific regions. There is also some limited support from mitochondrial gene sequence analysis; six *Nodilittorina* species were included as an outgroup in a study of *Littorina* (Reid et al., 1996), and the four members of *Echinolittorina* (all from the Atlantic) formed a clade. An early molecular study of Central American littorinids (Jones, 1972), based on allozymes and myoglobin, failed to unite seven species of *Nodilittorina*, but also did not demonstrate the integrity of five species of the undoubtedly monophyletic *Littoraria* (Reid, 1999a, b) and can therefore be disregarded.

Another question relating to the phylogeny and definition of *Nodilittorina* is the correct classification of the Atlantic species *Littorina* (*Littorina*) *striata*. This has been placed in *Nodilittorina* by some authors (Rosewater, 1981; Bandel & Kadolsky, 1982), although it appears as the basal extant species of *Littorina* (sister-group to *Nodilittorina*) in a species-level cladogram of that genus (Reid, 1996). Molecular evidence from both mitochondrial (Reid et al., 1996) and nuclear gene sequences (Winnepeinckx et al., 1998), and also from radular muscle proteins (Medeiros et al., 1998), support placement in *Littorina*.

Nevertheless, many uncertainties remain about the classification and phylogeny of this group. The genus is still not adequately defined by any unique morphological synapomorphy, and it may prove to be a paraphyletic or polyphyletic assemblage consisting of those littorinine species that do not fall into any of the other more well-defined genera (i.e., *Melarhaphe*, *Peasiella*, *Mainwarinia*, *Tectarius*, *Cenchritis*, *Littorina*). A particular concern is that none of the recent morphological or molecular analyses has included members of a group of *Nodilittorina* species from the southern oceans (considered part of subgenus *Nodilittorina* by Bandel & Kadolsky, 1982, and by Reid, 1989a; but here referred to the subgenus *Austrolittorina*, see Remarks on *N. fernandezensis*) that show some resemblances to *Littorina striata* (e.g., shape of pallial oviduct) as well as to typical *Nodilittorina* members (e.g., penis and paraspermatozoa). *Fossarilittorina* too is

a problematic group, with the Atlantic *N. meleagris* as type species (Rosewater, 1981). This is characterized by an unusual penis with closed sperm duct and no large glands, and its possible relationship to the eastern Pacific *N. modesta* and *N. porcata* groups remains unclear. Attempts have been made (Reid, unpublished) to include all recognized *Nodilittorina* species in a morphological phylogenetic analysis, but the results show an almost complete absence of structure, owing to relatively few informative characters and widespread homoplasy. These problems may only be resolved by means of new molecular studies, since the available morphological evidence is inadequate.

Meanwhile, the definition of *Nodilittorina* proposed by Reid (1989a) is followed here, although it is considered premature to assign the eastern Pacific species to any of the three constituent subgenera. The subgenus *Austrolittorina* is, however, used here for *N. fernandezensis*, to emphasize its closer relationship to *N. unifasciata*, the Australian type species of the subgenus (Rosewater, 1970), than to all the other eastern Pacific species. Unfortunately, the continuing uncertainty surrounding the phylogenetic relationships of *Nodilittorina* may, when resolved, have nomenclatural consequences. The type species of *Nodilittorina* was designated by Abbott (1954) as *N. pyramidalis*, an endemic Australian species which is in several respects atypical. Although its shell is nodulose (like such "typical" species as *N. dilatata* and *N. trochoides*), its pallial oviduct is identical to that of *N. (Austrolittorina) unifasciata*, and its penis has a papillose filament unique in the genus. It is possible that *N. pyramidalis* may prove to be a member of the *Austrolittorina* group, so that *Austrolittorina* may fall in the synonymy of *Nodilittorina*. If, in addition, it were to be discovered that the *Austrolittorina* group does not form a clade together with the other *Nodilittorina* species, the genus *Nodilittorina* (with *Austrolittorina* in synonymy) would have to be employed in a more restricted sense than at present.

In the following systematic account, the *Nodilittorina* species of the eastern Pacific are divided into three informal groups: six species in the *N. porcata* group, two in the *N. modesta* group, and six in the *N. aspera* group. This is convenient since the groups are easy to recognize morphologically. Furthermore, in each case, the included species are sufficiently similar that each group may well prove to be monophyletic. The relationships of the remaining four species are unclear and they are dealt with last.

### The *Nodilittorina porcata* Group

Considerable confusion has surrounded the identification and nomenclature of a group consisting of the following six species: *N. atrata*, *N. porcata*, *N. santelena*, *N. fuscolineata*, *N. parcipicta*, and *N. albicarinata*. All are small (less than 7.6 mm) and share a number of shell



features that are unusual in the genus *Nodilittorina*. Most striking is their protean plasticity of shell shape, sculpture, and coloration, which in some of these species equals that of any other littorinids. Once the species have been separated by anatomical criteria, it becomes clear that each shows a parallel and analogous range of shell variation. Thus shell shape ranges from globular to rather tall-spined and, while most show a perforated pseudo-umbilicus (or at least a crescentic area adjacent to the columella), this is closed or absent in juveniles and some adults. Shell sculpture consists of spiral microstriae, sometimes sufficiently regular and widely-spaced to be termed primary spiral grooves. Whereas some shells develop no further sculpture and therefore appear macroscopically smooth, others produce strong spiral ribs which, particularly at the periphery, may become flanged keels or carinae. Shell coloration is highly variable in all these species. Most can occur in an unpigmented white form, but more often develop a conspicuous pattern of brown or black axial stripes, spots, and spiral bands. In the Discussion it is argued that this striking variability in shell characters may be largely under ecophenotypic control. Certain anatomical characters are also shared by this group, including the absence of the penial glandular disc, peculiar twist to the end of the penial filament (slight or absent in two species), and flexure between straight and spiral portions of the pallial oviduct. These characters are likely synapomorphies and suggest that the group is a monophyletic one. They also share similar radulae, with pointed cusps; this, however, is characteristic of juvenile and small adult littorinids (see Reid, 1996, in *Littorina*) and may simply be an allometric effect of small size. Within the group, subjective similarities in shell, penes, and paraspermatozoa suggest two sub-groups, *N. atrata*, *N. porcata*, and *N. santelenae*, on one hand, and *N. fuscolineata*, *N. parcipicta*, and *N. albicarinata*, on the other.

Not surprisingly, this extreme intraspecific variation in shell shape has resulted in a confused taxonomy, at not only the specific but also the familial level. The earliest name is *Littorina porcata* Philippi, 1846, and this has continued to be widely applied to members of the group. In his catalogue of shells from Panama, C. B. Adams (1852a, b) described four species, of which only *atrata* was confidently placed in *Littorina* (then applied in a broad sense, including most members of the subfamily Littorininae); two of the remainder were doubtfully included, with the comment that they might be members of *Narica* (= *Vanikoro*, Vanikoroidae), while the fourth was doubtfully assigned to *Adeorbis* (Vitrinellidae). In fact, all are synonyms of *Nodilittorina atrata*, emphasizing its extreme variability. Succeeding authors were also uncertain about the familial assignment of these taxa and, on account of their usual possession of umbilicus and spiral carinae, often listed them as species of *Fossar* (H. & A. Adams, 1854) or *Fossarus* (A. Adams, 1855; Carpenter,

1857a, 1863, 1864a; Tryon, 1887; Turner, 1956; Keen, 1971; Abbott, 1974; Finet, 1985), a genus variously classified as littorinid, as a monotypic cerithioidean family and, most recently, as a member of the Planaxidae (Houbert, 1990). In recognition of this resemblance to *Fossarus*, Rosewater (1981) introduced *Fossarilittorina* as a subgenus of *Littorina* and included five names that have been applied to members of the *N. porcata* group (although the type species was the Atlantic *N. meleagris*). Bartsch & Rehder (1939) described a new taxon as a member of the littorinid genus *Peasiella* (see Reid, 1989b; Reid & Mak, 1998), misled by the trochoidal shell shape. The most widespread of the group, *N. atrata*, has also been misidentified as a species now assigned to *Iselica* (Pyramidellidae) and possibly as another now recognized as a *Tricolia* (Turbinidae) (see synonymy of *N. atrata*). In describing the new species *albicarinata*, McLean (1970) tentatively referred it to *Littorina*, and called for a detailed examination of the relationships of this eastern Pacific group with *Fossarus*. Their classifications as littorinids was finally established beyond doubt when Reid (1989a) described the general anatomy of *N. porcata* s.l. in a review of littorinid phylogeny, and placed it in the genus *Nodilittorina*.

At the specific level, members of the *N. porcata* group have been neglected by systematists. Only the species *porcata* was included in the nineteenth century monographs of *Littorina* (Philippi, 1847; Reeve, 1857; Wein-kauff, 1882; Tryon, 1887) and none was mentioned in von Martens' (1900) monograph of *Littorina* in Central America. Carpenter (1863) reviewed the taxa of C. B. Adams (1852a, b) from Panama, but reduced only one to synonymy. New taxa were added by A. Adams (1855), Carpenter (1864), Bartsch & Rehder (1939), and McLean (1970), and two additional species are described here. Keen (1971) figured "*Peasiella*" *roosevelti* and "*Littorina*" *albicarinata*, but other names in the group were simply listed uncritically as species of *Fossarus*. In Rosewater's (1970) list of worldwide Littorinidae, the only name that might apply to this group was *Littorina* (*Mel-larhappe*) *umbilicata* (this is doubtful; see Taxonomic History of *N. atrata*). In the only other recent attempt to list the species of Littorinidae, Reid (1989a) gave *N. porcata* (which included *N. atrata*) and *N. albicarinata*. Elsewhere in the systematic literature the members of the *N. porcata* group have appeared only in faunistic lists.

No critical revision has previously been attempted for these taxa and is only possible now owing to the availability of anatomical material. It was the diagnostic penial differences that provided the first intimation that six distinct species are involved. These differences in shape are subtle, but the interpretation is supported by their correlations with shell traits. Most importantly, each of the species is not only sympatric but also syntopic (i.e., occurring together in the same microhabitat) with at least one other in the group and, in such cases, the diagnostic



penial and shell traits are maintained. For those pairs not known to occur sympatrically, morphological differences are of the same order and, taken together with widely separated geographical ranges, support their specific status. Characters of the oviduct and radula are not generally useful for discrimination.

Identification of these species still poses a challenge, and the most useful features are summarized in Table 1. Since there is only partial overlap of their geographical ranges, and the number of sympatric taxa is not known to exceed three, information about geographical origin of samples is useful. If this is known, examination of shells is almost always adequate for identification. However, the critical characters are not usually the most obvious features such as shell outline, smooth or carinate sculpture, perforated or closed pseudo-umbilicus, or white as opposed to patterned surface. Instead, the details of color pattern and of microsculptural striation are more significant (Table 1). Penial characters can be used to confirm identification of male specimens, but even here, occasional problems are encountered if penes are strongly contracted or contorted before fixation, or when mamilliform penial glands are missing (a rare abnormality, seen in two of the approximately 100 males examined).

In addition to their morphological similarity, the six members of the *N. porcata* group share some similarity in their ecology. Whereas most species of *Nodilittorina* are typically found among the superficially bare rocks and crevices of the littoral fringe, extending down to the uppermost eulittoral, those of the *N. porcata* group are generally to be found at a lower level, among barnacles, in crevices, and in shallow pools, in the mid to upper eulittoral zone. Whether this is simply a reflection of their small size, or of some physiological or dietary characteristic, is unknown.

*Nodilittorina atrata* (C. B. Adams, 1852)

(Figures 1A–I, 3A–G, 4A, F, G, H, 5A, 6)

*Littorina atrata* C. B. Adams, 1852a:395–396, 537 (Panama; lectotype (Turner, 1956) MCZ 186444, seen, Turner, 1956:pl. 9, fig. 5, Figure 1E herein; approx. 200 paralectotypes MCZ 186445, seen; 26 paralectotypes BMNH 1865.11.22.92, seen; 9 paralectotypes BMNH 1865.11.24.181, seen). C. B. Adams, 1852b:171–172, 313. Carpenter, 1857a:273. Carpenter, 1863:352. Vermeij, 1973:324.

*Littorina atrata*—Carpenter, 1857a:326.

*Fossarus atratus*—Carpenter, 1863:352. Tryon, 1887:272, pl. 52, fig. 10. Hertlein & Strong, 1939:371. Morrison, 1946:10–11. Turner, 1956:33, pl. 9, fig. 5. Keen, 1971:454, fig. 772.

*Fossarina atrata*—Carpenter, 1864b:550.

*Littorina (Fossarilittorina) atrata*—Rosewater, 1981:30. Finet, 1985:13.

*Littorina (?) excavata* C. B. Adams, 1852a:396, 537 (Panama; holotype MCZ 186422, seen, Turner, 1956:pl. 13,

fig. 2). C. B. Adams, 1852b:172, 313. Carpenter, 1857a:273.

*Fossar excavatus*—H. & A. Adams, 1854:320.

*Fossarus excavatus*—Carpenter, 1857a:326. Carpenter, 1863:352. Pilsbry & Lowe, 1932:124. Turner, 1956:47, pl. 13, fig. 2. Keen, 1971:454.

*Littorina (Fossarilittorina) excavata*—Rosewater, 1981:30. Finet, 1985:13.

*Littorina (?) foveata* C. B. Adams, 1852a:397, 537 (Panama; lectotype (Turner, 1956) MCZ 186454, seen, Turner, 1956:pl. 9, fig. 6; 1 paralectotype lost). C. B. Adams, 1852b:173, 313. Carpenter, 1857a:273.

*Fossar foveatus*—H. & A. Adams, 1854:320.

*Fossarus foveatus*—Carpenter, 1857a:326. Carpenter, 1863:352. Turner, 1956:50–51, pl. 9, fig. 6. Keen, 1971:454.

*Adeorbis (?) abjecta* C. B. Adams, 1852a:407–408, 539 (Panama; lectotype (Turner, 1956) MCZ 186338, seen, Turner, 1956:pl. 9, fig. 7; 15 paralectotypes MCZ 186339, seen). C. B. Adams, 1852b:183–184, 315. Carpenter, 1857a:273. Turner, 1956:27, pl. 9, fig. 7.

*Fossar abjectus*—H. & A. Adams, 1854:320.

*Fossarus abjectus*—Carpenter, 1857a:326. Carpenter, 1863:354. Hertlein & Strong, 1955a:137. Keen, 1971:453. Finet, 1985:17.

*Lacuna abjecta*—Pilsbry & Lowe, 1932:124.

*Fossar variegatus* A. Adams, in H. & A. Adams, 1854:319–320; 3:pl. 33, fig. 7a, b (operculum) (*nomen nudum*).

*Fossar variegatus* A. Adams, 1855:187 (Eastern Seas [in error, here corrected to Panama]; 4 syntypes BMNH 1968821, seen).

*Fossarina variegata*—Nevill, 1885:171.

*Littorina porcata*—Nevill, 1885:138 (not Philippi, 1846).

*Nodilittorina (Nodilittorina) porcata*—Reid, 1989a:100, fig. 5h (in part, includes *N. porcata*). Skoglund, 1992:15, 16, 33, 34 (in part, includes *N. porcata*).

*Fossarus angiosomus*—Morrison, 1946:10 (not *Littorina angiosoma* C. B. Adams, 1852, which is an *Iselica*, Pyramidellidae; personal observation of lectotype MCZ 186442). Hertlein & Strong, 1955a:137 (not C. B. Adams, 1852). Finet, 1985:17 (as *F. angiosoma*; not C. B. Adams, 1852).

*Littorina (Melarhaphé) ? angiosoma*—Jones, 1972:2 (not C. B. Adams, 1852).

*Littorina angiosoma*—Rosewater, 1980:7, figs. 7, 8 (radula) (not C. B. Adams, 1852).

*Littorina (Fossarilittorina) angiosoma*—Rosewater, 1981:30 (not C. B. Adams, 1852).

*Nodilittorina (Nodilittorina) angiosoma*—Skoglund, 1992:15. Kaiser, 1993:106. Kaiser, 1997:27. (All not *Littorina angiosoma* C. B. Adams, 1852).

*Nodilittorina angiosoma*—Finet, 1994:18 (not *Littorina angiosoma* C. B. Adams, 1852).

? *Littorina (?) Melarhaphé umbilicata*—Rosewater, 1970:424 (not *Littorina umbilicata* d'Orbigny, 1840, which is a *Tricolia*, see Keen, 1971:358; personal observation of holotype in BMNH).

? *Littorina umbilicata*—Alamo & Valdivieso, 1987:26. Alamo & Valdivieso, 1997:18. Paredes, Huamán, Cardoso, Vivar & Vera, 1999:22. (All not d'Orbigny, 1840).

See also Synonymy and Taxonomic History of *N. porcata*.

**Taxonomic history:** The history of this species is one of considerable complexity, involving uncertain generic as-

Table 1  
Summary of the most useful characters for the identification of the six species of the *Nodilittorina porcata* group.

Character	<i>N. atrata</i>	<i>N. porcata</i>	<i>N. santelmae</i>	<i>N. fuscolineata</i>	<i>N. parvipicta</i>	<i>N. albicarinata</i>
1. Geographical range	El Salvador to N Peru, Isla del Coco, Galápagos Is	Galápagos Is	S Ecuador, N Peru	El Salvador to S Ecuador	S Baja California, Sinaloa to Michoacán (Mexico)	SW Baja California, Gulf of California
2. Shell						
—pseudo-umbilicus	present	present	absent	present	present	absent
—smooth form	yes	yes	yes	rare	no	yes
—carinate form	yes	peripheral carina only	rare	strong ribs only	yes	yes
—all white form	yes	yes	no	no	rare	yes
—most common pattern	broad black and white stripes from suture to base, with black band above periphery	dark marbling or fine axial stripes, with spots on basal ribs	brown with white band at suture and base	pale with brown lines or long dashes on ribs	small brown spots on all ribs	brown with white carinae
3. Penis						
—filament tip	pointed, twisted	pointed or hooked, twisted	slightly pointed, twisted	pointed, not twisted	blunt, not twisted	pointed, twisted
—mamilliform gland	moderately large, on projection of base	moderately large, attached directly to base	small, on projection of base	small, attached directly to base	large, attached directly to base	large, on projection of base
—pigment on base	absent	sometimes present	sometimes present	sometimes present	sometimes present	present

signment, synonyms, misidentification, and confusion with other members of a complex of similar species. In the account of the shells of Panama in which C. B. Adams (1852a, b) first described this species, he introduced three additional taxa which prove to be its synonyms, a measure of the great intraspecific variability of this species. *Littorina atrata* included largely black shells with small umbilicus, although he noted its variability in shape and sculpture. *Littorina foveata* was based upon patterned shells with a wide umbilicus, and *L. excavata* referred to the smooth, globular white form (as in Figure 1H), of which Adams had only a single specimen. These latter two were both only doubtfully assigned to *Littorina*, and Adams suggested that *Narica* (= *Vanikoro*) might be more suitable. The last taxon, *Adeorbis abjecta*, was based upon shells with a low, eroded spire; again the generic assignment was tentative; and Adams noted a resemblance to "*Littorina*" *porcata*; Carpenter (1863) placed it in the synonymy of "*Fossarus*" *atratus*. For almost 100 years these names were scarcely mentioned in the literature, except in the reviews of Carpenter (1857a, 1863) and iconography of Tryon (1887), and there as species of *Fossarus*. During the middle part of the twentieth century, studies of West American mollusks increased, and these supposed *Fossarus* species appeared in several faunistic lists (Pilsbry & Lowe, 1932; Hertlein & Strong, 1939; Morrison, 1946). The types of C. B. Adams were figured by Turner (1956), but even Keen (1971) simply listed them, unfigured, as *Fossarus* species. That they were in fact littorinids was eventually recognized by Rosewater (in Jones, 1972; Rosewater, 1980, 1981), and Vermeij (1973), who used the genus *Littorina*, and confirmed by Reid (1989a), who used *Nodilittorina*. Another synonym is *Fossar variegatus*, an almost entirely neglected name. This was based on material in the Cuming Collection, but in his description A. Adams (1855) gave the incorrect locality "Eastern Seas"; the four syntypes are somewhat eroded, but are clearly examples of a form of *N. atrata* that is common in Central America, and could easily have been collected by Cuming during his travels in the region.

Hitherto, no authors have discriminated among all six members of the *N. porcata* complex, and there has therefore been much misidentification, although geographical distribution can sometimes be used to recognize the species intended. Since *porcata* Philippi, 1846 is the oldest name, it has been the most widely used in the past decade (following Reid, 1989a; see Skoglund, 1992; Kaiser, 1993, 1997; Finet, 1994). However, both *N. atrata* and *N. porcata* occur together in the Galápagos Islands, and so the names used in faunistic lists of Galápagos species (Stearns, 1893b; Hertlein & Strong, 1939, 1955a; Vermeij, 1973; Kaiser, 1993, 1997; Finet, 1985, 1994) cannot be confidently assigned in the synonymies. To add to the confusion, the name *angiostoma* also appears frequently in the literature. This is a misidentification; *Littorina an-*

*giostoma* C. B. Adams, 1852, is an *Iselica*, belonging to the Pyramidellidae (figured by Turner, 1956:pl. 13, fig. 1). This name has been used mainly in works on the Galápagos fauna (Hertlein & Strong, 1955a; Finet, 1985, 1994; Kaiser, 1993, 1997), and these also list at least one other species (usually *porcata* or *abjecta*). It seems likely that *angiostoma* was used for shells of the white ecotype, and *porcata* for patterned shells. However, this distinction does not separate *N. atrata* s.s. and *N. porcata* s.s. in the Galápagos, so that again the synonymy cannot be resolved. For convenience, in these doubtful cases, uses of the name *atrata* and its synonyms (including *angiostoma* non C. B. Adams) for Galápagos shells are listed above, while uses of *porcata* (and its synonym *roosevelti*) are given in the synonymy of *N. porcata*.

Curiously, in his influential work on Indo-Pacific Littorinidae in which all species recognized worldwide were listed, Rosewater (1970) did not give any of the names discussed above. Instead, the older name *Littorina umbilicata* appears, doubtfully assigned to the subgenus *Melanhaphe*. This may have been intended to refer to some of the members of the *N. porcata* group, although the distribution given ("Peru and Chili," perhaps following Dall, 1909) is not correct (*N. atrata* and *N. santelenae* only just reach far northern Peru). If so, this is another misidentification, since *Littorina umbilicata* d'Orbigny, 1840, is in fact a species of *Tricolia* (Keen, 1971). The name also appears in several recent lists of Peruvian mollusks (Alamo & Valdivieso, 1987, 1997; Paredes et al., 1999), in which *Tricolia umbilicata* is listed separately, suggesting that "*L. umbilicata*" might indeed be intended for *N. atrata* and/or *N. santelenae*.

**Diagnosis:** Shell small, globular to tall, smooth to carinate; coarse, irregular microstriae; usually a large, perforated, pseudo-umbilicus; may be white, but common pattern is broad black or brown axial stripes from suture to base and broad band above periphery, on white ground. Penis with pointed and twisted filament tip, moderately large mamilliform gland on long projection of base, no glandular disc.

**Material examined:** 73 lots (including 26 penes, 11 sperm samples, eight pallial oviducts, one egg capsule, five radulae).

**Shell (Figures 1A–I):** Mature shell height 2.1–7.5 mm. Shape variable; high turbinate to low-spined, globular or slightly patulous ( $H/B = 1.00\text{--}1.53$ ;  $SH = 1.24\text{--}2.21$ ); spire whorls rounded, suture distinct; periphery of last whorl usually rounded, but may be marked by a rib or carina; solid. Columella straight, narrow, flared and flattened at base; pseudo-umbilicus usually large, perforated, outlined by sharp keel continuous with outer apertural lip, but sometimes only narrow imperforate crescentic area (pseudo-umbilicus narrow or absent in most juveniles).

Sculpture variable; smoothest shells covered with coarse, rather irregular, spiral microstriae (rarely becoming obsolete above periphery of last whorl); sometimes 5–16 indistinct ribs may develop on last whorl (peripheral rib and 2–3 on base are strongest); strongly sculptured shells with few sharp or carinate ribs, 2–3 on base, strongest rib at periphery, 1–7 above periphery (juveniles 2 on base, 1 at periphery, 1 at shoulder), entire surface with strong microstriae; periostracum occasionally produced into minute bristles (to 100  $\mu\text{m}$ ) on basal and peripheral ribs of strongly sculptured shells. Protoconch 0.26–0.29 mm diameter. Color variable, may change abruptly; frequently entirely white externally (especially in smooth, globular shells), sometimes with irregular large blotches of black, fine brown speckles, or continuous dark brown band between shoulder and periphery; most common color is striking black and white pattern, with broad, black or dark brown oblique axial stripes from suture to base, usually fused between shoulder and periphery to form irregular continuous dark band; rarely entirely black but for few white flames on base; columella and aperture usually pink-brown to dark purple-brown (even in white shells), with anterior and often posterior unpigmented band.

**Animal:** Head and sides of foot grey to black; 2 black lines along tentacle, seldom meeting at tip; usually a narrow white band across snout. Opercular ratio 0.41–0.47. Penis (Figures 3A–G): filament tip pointed and twisted; sperm groove with a kink, distal portion shallower, extending to filament tip; single mamilliform penial gland on long lateral appendage at 0.4–0.6 total penial length (mamilliform gland absent in one specimen); glandular disc absent; base not pigmented. Euspermatozoa 79–107  $\mu\text{m}$ ; paraspermatozoa (Figures 4G, H) oval, 14–26  $\mu\text{m}$ ; rod-pieces single (rarely two), filling cell or projecting at one end (rarely both ends), 14–21  $\mu\text{m}$ , blunt or slightly rounded at ends, parallel-sided or slightly tapering; granules large, spherical, distinct. Pallial oviduct (Figure 4A) with flexure and constriction between spiral and straight sections; copulatory bursa opening at anterior end of straight section, extending back to start of spiral portion. Spawn (Figure 4F) a pelagic cupola-shaped capsule 160  $\mu\text{m}$  diameter, sculptured with four concentric rings, containing single ovum 40  $\mu\text{m}$  diameter; protoconch indicates planktotrophic development.

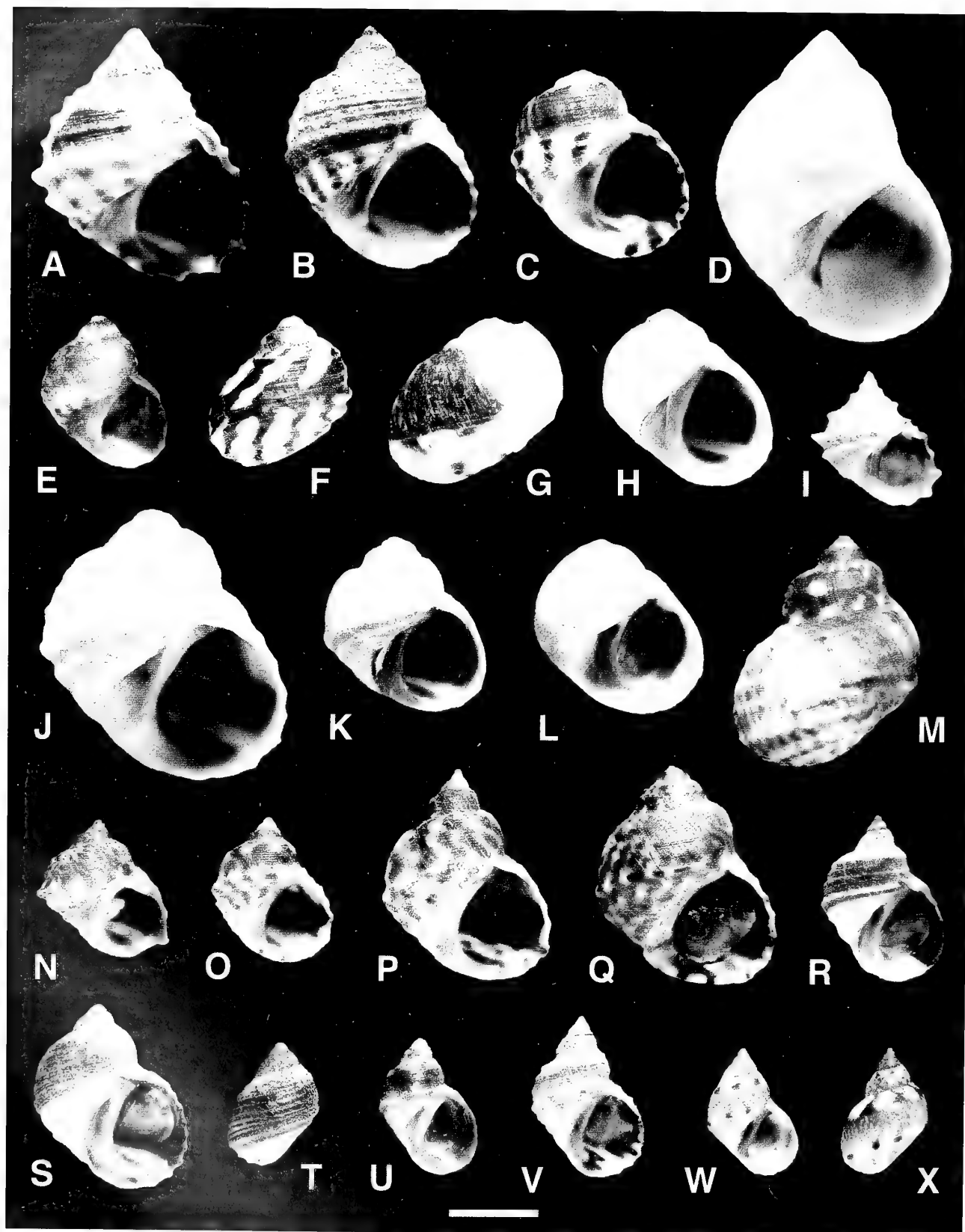
**Radula (Figure 5A):** Relative radular length 2.1–3.5. Rachidian: length/width 1.11–1.68; major cusp pointed and elongate leaf-shaped. Lateral and inner marginal: major cusps pointed or slightly rounded. Outer marginal: 5–7 cusps.

**Habitat:** Among uppermost oysters and barnacles; in crevices and shallow pools in mid to upper eulittoral; on sandstone, basalt, concrete; sheltered to exposed coasts, sometimes in silty, mangrove-fringed channels; often abundant.

**Range (Figure 6):** El Salvador to northern Peru, Isla del Coco, Galápagos Islands. Range limits: Los Cobanos, Sonsonate, El Salvador (LACM 73-56, 3 specimens); Isla Zacatillo, Golfo de Fonseca, El Salvador (LACM 73-57); Coyolita, Honduras (USNM 749644); San Juan del Sur, Nicaragua (USNM 60677); Puerto Utria, Choco, Colombia (LACM 34-106.20, 1 specimen); Same, Esmeraldas, Ecuador (BMNH 2001151); El Rubio and Punta Mero, Tumbes, Peru (LACM 72-85, 1 specimen); Isla del Coco (USNM 130103; KKK); Galápagos Islands (Isla Santiago, USNM 807236; Isla Floreana, personal observation; Isla San Cristobal, BMNH 20001152; Isla Bartolomé, BMNH 20001153; Isla Santa Cruz, BMNH 20001154). The record from Los Cobanos is an isolated patch of hard substrate on a largely sedimentary coast, the Central American Gap (Glynn & Ault, 2000). The species is common in the Golfo de Fonseca, Isla del Coco, the Galápagos Islands and elsewhere, but only single specimens have been seen from Colombia and Peru.

**Remarks:** The shell of *N. atrata* is among the most variable of all littorinid species, ranging from globular to tall, umbilicate to imperforate, smooth to carinate, white to black-patterned. There is no apparent geographical component to this variation; for example, the distinctive smooth, white, globular shells are recorded from the Galápagos Islands, Ecuador, Panama, and Costa Rica. However, it is notable that of the available museum collections, most individual samples encompass a relatively restricted range of shell variation, and extreme variability in color or sculpture from a single locality is unusual. Personal collections suggest that there may be a correlation with microhabitat, though this requires further investigation. For example, at Punta Chocollatera (Peninsula Santa Elena, Guayas, Ecuador) a sample from crevices among barnacles in the upper eulittoral comprised ribbed shells, mostly with strong black patterning (BMNH 20001155, Figure 1F), whereas a sample from shallow pools on an open rock platform only 20 m distant comprised only white, smooth, globular shells (BMNH 20001156, similar to Figure 1H). A similar, but less perfect, correlation was observed nearby at Anconcito (BMNH 20001157, 20001158). In general, samples from lower tidal levels (mid eulittoral) and from pools appear to be more smooth and sometimes white in color, whereas those from among barnacles are more strongly ribbed or carinate. Shell color may change abruptly on a single individual, for example from black patterned to entirely white or vice versa (Figure 1G). Sculpture does not show similarly sudden change, although relatively smooth shells sometimes develop low ribs on the final part of the last whorl. A possible interpretation of these observations is that the species are susceptible to ecophenotypic effects on shell form and coloration (see Discussion).

This is the most widely distributed of the six species in the *N. porcata* group. It is sympatric with *N. fuscoli-*



*neata* from El Salvador to Ecuador, with *N. porcata* in the Galápagos Islands, and with *N. santelenae* in southern Ecuador and northern Peru. In each case, these can be found in the same microhabitat as *N. atrata*, although some differences in habitat range are likely (see Remarks on all three). These four potentially sympatric species can be distinguished by penial shape but, owing to the great range of variation, identification from shells alone is sometimes difficult (Table 1). In the Galápagos Islands both *N. atrata* and *N. porcata* may be white; the latter usually shows fine, regular microstriae, an angled periphery, and a strong peripheral and two basal ribs; in *N. atrata* the microstriae are coarser and more irregular, the periphery rounded and, if ribs are present at all, they are uniformly developed over the whorl. Patterned shells are more distinctive; in *N. atrata*, the common black and white pattern of broad axial stripes from suture to base and broad dark band above the periphery is diagnostic; dark shells of *N. porcata* are usually marbled or finely striped, with brown spots on the basal ribs and often a pale basal band. In southern Ecuador and northern Peru, shell coloration again usually distinguishes *N. atrata* from sympatric *N. santelenae*; the latter is commonly brown or black with a white band on the base and often a second at the suture, or (in the smooth algal-dwelling form) mottled yellow-brown with strong brown and white spots at shoulder and periphery. The shell of *N. fuscolineata* is smaller, more delicate, with markedly rounded whorls and regular ribs bearing a pattern of dark lines or dashes.

*Nodilittorina porcata* (Philippi, 1846)

(Figures 1J–Q, 3H–O, 4B, I, 5B, 6, 22E)

*Littorina porcata* Philippi, 1846a:139 (ad insulas Gallapagos [Galápagos Islands, Ecuador]; lectotype (here designated, 6.1 × 5.0 mm) BMNH 1968218/1, seen, Philippi, 1847:3, *Littorina* pl. 6, fig. 14, Figure 1J herein; 1 paralectotype BMNH 1968218/2, seen; 2 probable paralectotypes BMNH 1998193, seen). Carpenter, 1857a:186. Reeve, 1857:sp. 89, pl. 16, fig. 89. Stearns, 1893b:444. *Littorina porcata*—Philippi, 1847:3:14, *Littorina* pl. 6, fig.

14. Carpenter, 1857a:326, 360. Weinkauff, 1882:78–79, pl. 10, fig. 12. Weinkauff, 1883:215.

*Littorina (Littorina) porcata*—Tryon, 1887:242, pl. 41, fig. 10.

*Fossarus porcatus*—Keen, 1971:454, fig. 780.

*Littorina (Fossarilittorina) porcata*—Rosewater, 1981:30. Finet, 1985:13.

*Nodilittorina (Nodilittorina) porcata*—Reid, 1989a:100 (in part, includes *N. atrata*). Reid, 1989b:53. Skoglund, 1992:15, 16, 33, 34 (in part, includes *N. atrata*). Kaiser, 1993:106. Kaiser, 1997:27.

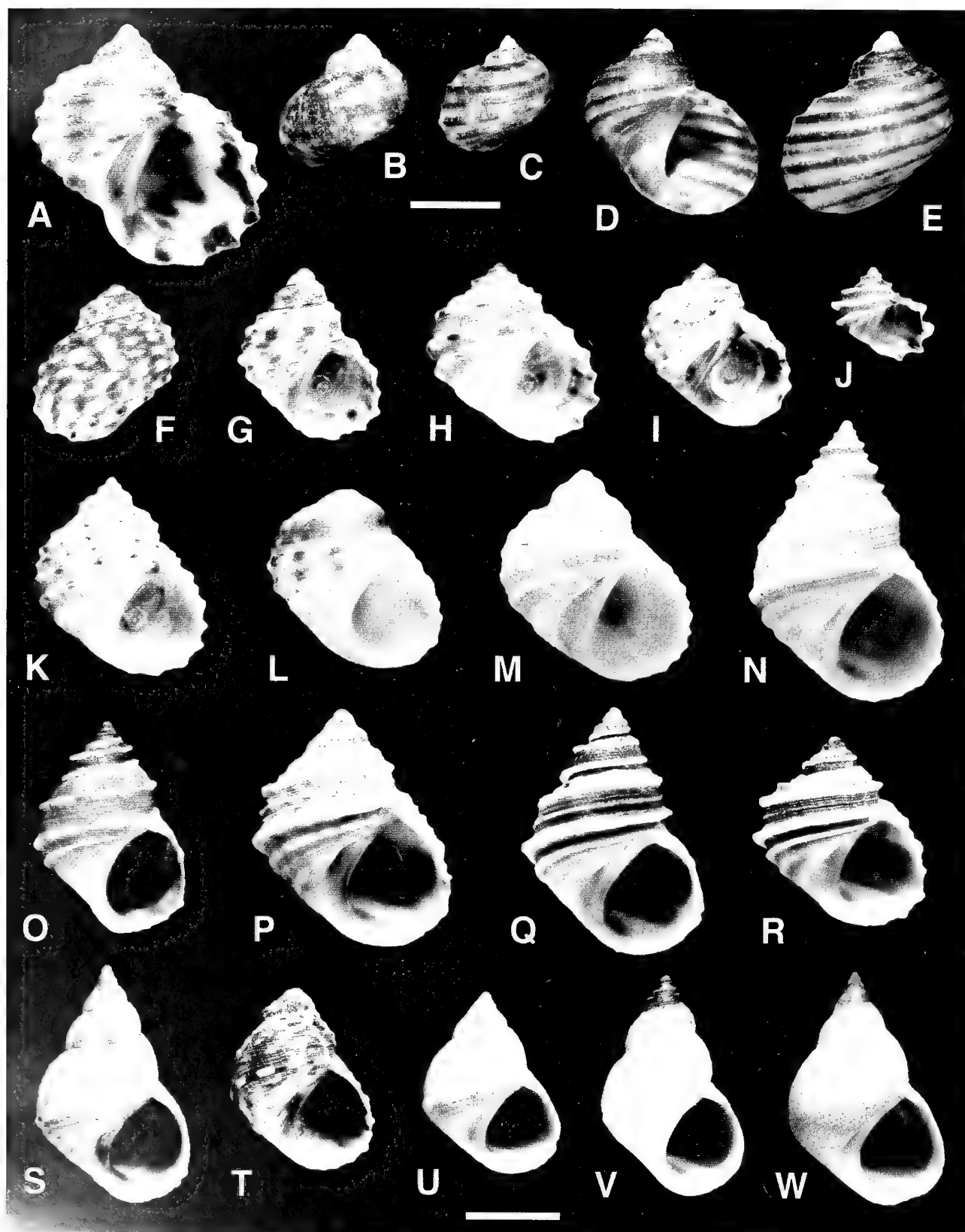
*Nodilittorina porcata*—Finet, 1994:18 (in part, includes *N. atrata*).

*Peasiella roosevelti* Bartsch & Rehder, 1939:8–9, pl. 2, figs. 1–3 (Sullivan [Sullivan] Bay, James Island [Isla Santiago], Galápagos; holotype USNM 472575, seen, Figure 1N herein). Keen, 1971:367, fig. 191. Finet, 1985:13.

See also Synonymy and Taxonomic History of *N. atrata*.

**Taxonomic history:** The types of *Littorina porcata* are large and white, with variably developed ribs on the last whorl. In contrast, *Peasiella roosevelti* was based on a single, small, darkly patterned shell with strong peripheral keel. Surprisingly, in view of the longstanding assignment of other members of the *N. porcata* group to the genus *Fossarus*, Philippi (1846a) immediately classified his species as a member of *Littorina*, and it was subsequently included in several monographs of the genus (Philippi, 1847; Reeve, 1857; Weinkauff, 1882; Tryon, 1887); only Keen (1971) referred it to *Fossarus*. The trochoidal shape and umbilicus of the form described by Bartsch & Rehder (1939) are indeed reminiscent of the littorinid genus *Peasiella*, but that has a multispiral operculum (Reid, 1989b; Reid & Mak, 1998), a feature missing from the type, which was inhabited by a pagurid crab. Since *porcata* is the oldest name in the *N. porcata* group, it has been frequently misapplied to the more widespread *N. atrata*. These two very similar species occur together on the Galápagos Islands, and previous authors working on the fauna have not distinguished them, so that synonymy

Figure 1. Shells of *Nodilittorina porcata* group: *N. atrata* (A–I), *N. porcata* (J–Q), and *N. santelenae* Reid, sp. nov. (R–X). A. Isla Pedro González, Panama (USNM 587820). B. Punta Morales, Golfo de Nicoya, Costa Rica (BMNH 20001159). C. Punta Pitt, Isla San Cristobal, Galápagos Islands (BMNH 20001152). D. Baltra Channel, Isla Santa Cruz, Galápagos Islands (BMNH 20001160). E. Lectotype of *Littorina atrata* C. B. Adams, 1852; Panama (MCZ 186444). F. Punta Chocolatera, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20001155). G. Punta Egas, Isla Santiago, Galápagos Islands (USNM 807236). H. Montezuma, Costa Rica (USNM 664373). I. Muisne, Esmeraldas, Ecuador (BMNH 20001161). J. Lectotype of *Littorina porcata* Philippi, 1846; Galápagos Islands (BMNH 1968218/1). K. Puerto Ayora, Isla Santa Cruz, Galápagos Islands (KLK). L, M, O–Q. Puerto Ayora, Isla Santa Cruz, Galápagos Islands (L, BMNH 20001167; M, Q, BMNH 20001164; O, P, BMNH 20001168). N. Holotype of *Peasiella roosevelti* Bartsch & Rehder, 1939; Bahía Sullivan, Isla Santiago, Galápagos Islands (USNM 472575). R. Holotype of *N. santelenae* Reid, sp. nov.; Punta Carnero, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20000309). S, T. Paratypes of *N. santelenae* Reid, sp. nov.; Punta Carnero, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20000310). U. Salinas, Guayas, Ecuador (USNM 368112). V. Punta Chocolatera, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20001171). W, X. Anconcito, Guayas, Ecuador (BMNH 20001170). Scale bar = 2 mm.





mies cannot be accurately compiled (see Synonymy and Taxonomic History of *N. atrata*).

**Diagnosis:** Shell small, globular to turbate; peripheral rib or carina, smooth or ribbed above; fine, regular microstriae; large, perforated pseudo-umbilicus; often white; if patterned, then dark marbling or fine stripes above periphery, spots on base. Penis with pointed or hooked, twisted filament tip, moderately large mamilliform gland closely attached to base, no glandular disc.

**Material examined:** 30 lots (including 19 penes, 1 sperm sample, 2 pallial oviducts, 3 radulae).

**Shell (Figures 1J–Q, 22E):** Mature shell height 1.9–6.1 mm. Shape variable; turbate to low-spined and globular ( $H/B = 1.04\text{--}1.26$ ,  $SH = 1.29\text{--}1.83$ ); spire whorls rounded, with distinct suture, often appearing turreted (owing to shoulder angulation and strong peripheral rib just above suture); last whorl usually with angulate periphery marked by strong rib or carina, sometimes rounded but still with peripheral rib; solid. Columella straight, narrow, flared, and flattened at base; pseudo-umbilicus large, perforated (narrow or imperforate in juveniles), outlined by sharp keel continuous with outer apertural lip. Sculpture variable; usually sharp or carinate peripheral rib and 2 basal ribs; smooth above periphery or with 1–6 rounded ribs developing on last whorl; occasionally all but peripheral rib become obsolete; fine, regular spiral microstriae usually present over entire surface (Figure 22E), but globular shells lacking ribs may be microscopically smooth; periostracum occasionally produced into fine bristles (to 200  $\mu\text{m}$ ; Figure 22E) on basal and peripheral ribs. Protoconch not seen. Color variable, may change abruptly; white or grey with brown to black oblique or wavy axial stripes, or fine marbling or irregular marks; base with brown spots on ribs, and often a pale spiral band on rib anterior to periphery; color pattern may become paler or disappear toward end of last whorl; shells may appear entirely white, although spire usually shows dark pattern unless heavily eroded; aperture dark brown or orange-brown, with anterior (sometimes also posterior) unpigmented band, columella brown.

**Animal:** Head and sides of foot black; two thick black lines along tentacle, usually meeting at tip. Opercular ratio 0.42–0.53. Penis (Figures 3H–O): filament tip pointed and twisted, often giving hooked appearance; sperm groove with a kink, distal portion shallower, extending to filament tip; single mamilliform penial gland closely attached to base at 0.2–0.4 total penial length; glandular disc absent; base often slightly pigmented. Euspermatozoa length unknown; paraspermatozoa (Figure 4I) oval; rod-pieces single (rarely two), projecting at one or both ends, 11–19  $\mu\text{m}$ , blunt, slightly tapering; granules large, spherical, distinct. Pallial oviduct (Figure 4B) with marked flexure and constriction between spiral and straight sections; copulatory bursa opening at anterior end of straight section, extending back to start of spiral portion. Spawn and development not observed.

**Radula (Figure 5B):** Relative radular length 1.3–1.8. Rachidian: length/width 0.94–1.27; major cusp pointed and elongate leaf-shaped. Lateral and inner marginal: major cusps pointed. Outer marginal: 6–7 cusps.

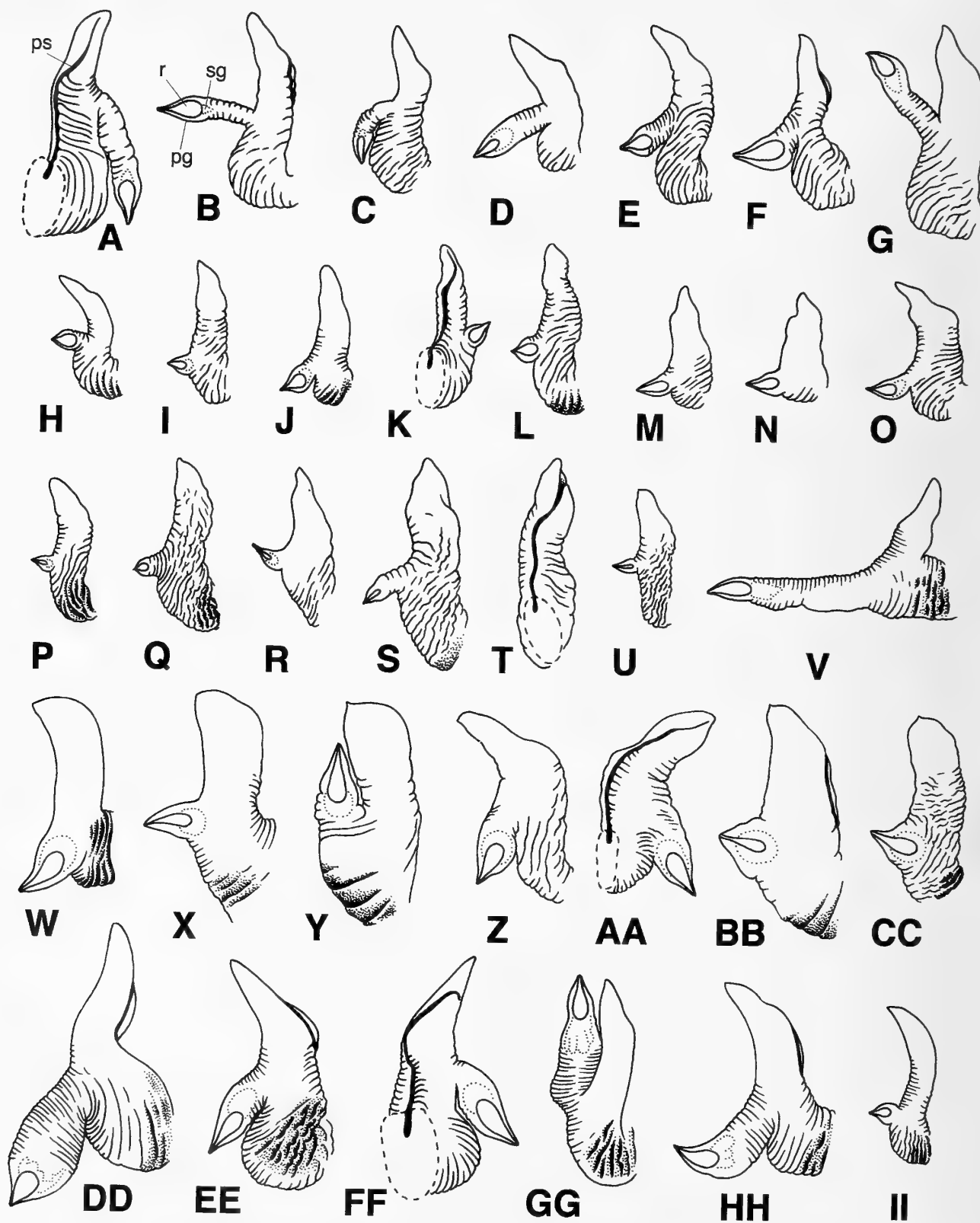
**Habitat:** In pits and shallow pools on basalt rocks and rock platforms, also concrete; upper eulittoral, sheltered to semi-exposed coasts; often abundant. In the Galápagos, *N. porcata* has also been reported from mangroves (Hedgpeth, 1969; Kay, 1991), but whether this refers to this species, *N. atrata* or both, is unknown.

**Range (Figure 6):** Galápagos Islands only. Records from islands of Santa Cruz (BMNH 20001164), San Cristobal (BMNH 20001165), Bartolomé (BMNH 20001166), Santiago (USNM 807236), Fernandina (LACM 72-196), Isabela (LACM 33-163, 34-276), Baltra (LACM 66-206), and Genovesa (LACM 33-174).

**Remarks:** This species is highly variable in shape, sculpture, and coloration. The two named taxa represent extremes of the range of variation; the types of *Littorina porcata* are large, smooth or ribbed, and white, whereas the type of *Peasiella roosevelti* is small, sharply keeled, and darkly patterned. However, there seems no doubt that these are conspecific. Sculpture above the periphery varies from smooth to ribbed within some microsympatric

←

Figure 2. Shells of *Nodilittorina porcata* group (continued): *N. fuscolineata* Reid, sp. nov. (A–E), *N. parcipicta* (F–M), and *N. albicarinata* (N–W). A. Holotype of *N. fuscolineata* Reid, sp. nov.; Punta Carnero, Peninsula Santa Elena, Guayas, Ecuador (BMNH 19990422). B. Isla San Pedrito, Costa Rica (LACM 72-22). C. Punta Penca, Guanacaste, Costa Rica (LACM 72-38). D, E. Isla Alcatraz, Costa Rica (LACM 72-46; two views). F. Mazatlán, Sinaloa, Mexico (BMNH 20001177). G. Boca de Tomatlán, Jalisco, Mexico (BMNH 20001178). H, I, K. 7 km NE of San José del Cabo, Baja California Sur, Mexico (BMNH 20001179). J. Playa de los Muertos, Puerto Vallarta, Jalisco, Mexico (BMNH 20001180). L. Lectotype of *Fossarus parcipictus* Carpenter, 1864; Cabo San Lucas, Baja California Sur, Mexico (USNM 4060). M. Topolobampo, Sinaloa, Mexico (BMNH 20001176). N, P–R. Balandra, Baja California Sur, Mexico (BMNH 20001182). O. Holotype of *Littorina albicarinata* McLean, 1970; El Requeson, Bahía Concepción, Baja California Sur, Mexico (LACM 1399). S. Puerto Lobos, Sonora, Mexico (USNM 862206). T. Playa Coromuel, Baja California Sur, Mexico (BMNH 20001186). U–W. Bahía Agua Verde, Baja California Sur, Mexico (USNM 264989). Scale bars A–E = 1 mm; F–W = 2 mm.



collections, and ribbed specimens usually show smooth spires. Spire height too varies substantially within single samples. The variation in shell color is peculiar, since available samples are either white or darkly patterned, and not a mixture of both. Samples collected from the same locality in different years can differ strikingly in coloration; for example, shells from the precise location of basalt rocks beside the dock of the Charles Darwin Research Station in Academy Bay, Santa Cruz Island, were white (and smooth) in collections made in 1988 and 1989, but brown (and ribbed) in 1998 (personal observation; BMNH 20001164, 20001167; CDRS). Close examination of white shells with well preserved spires shows that the early whorls are in fact brown. Furthermore, adults with predominantly brown shells can be found in which the coloration abruptly becomes pale or white on the last whorl (Figure 1M). As in some other members of the *N. porcata* group, these observations suggest that shell color (and perhaps also sculpture) may be influenced by ecophenotypic effects (see Discussion).

In museum collections from the Galápagos Islands, this species is often mixed with the closely similar *N. atrata*, and the two are syntopic in the habitats described above (personal observation). Nevertheless, there is probably some microhabitat or behavioral segregation between them, since in samples from Academy Bay, Santa Cruz Island, brown shells of *N. porcata* were frequently overgrown with a fine filamentous alga, whereas white shells of *N. atrata* from the same shores were not. The discrimination of these two species is discussed in the Remarks on *N. atrata*.

*Nodilittorina santelenae* Reid, sp. nov.

(Figures 1R–X, 3P–U, 4C, J, K, 5C, 6)

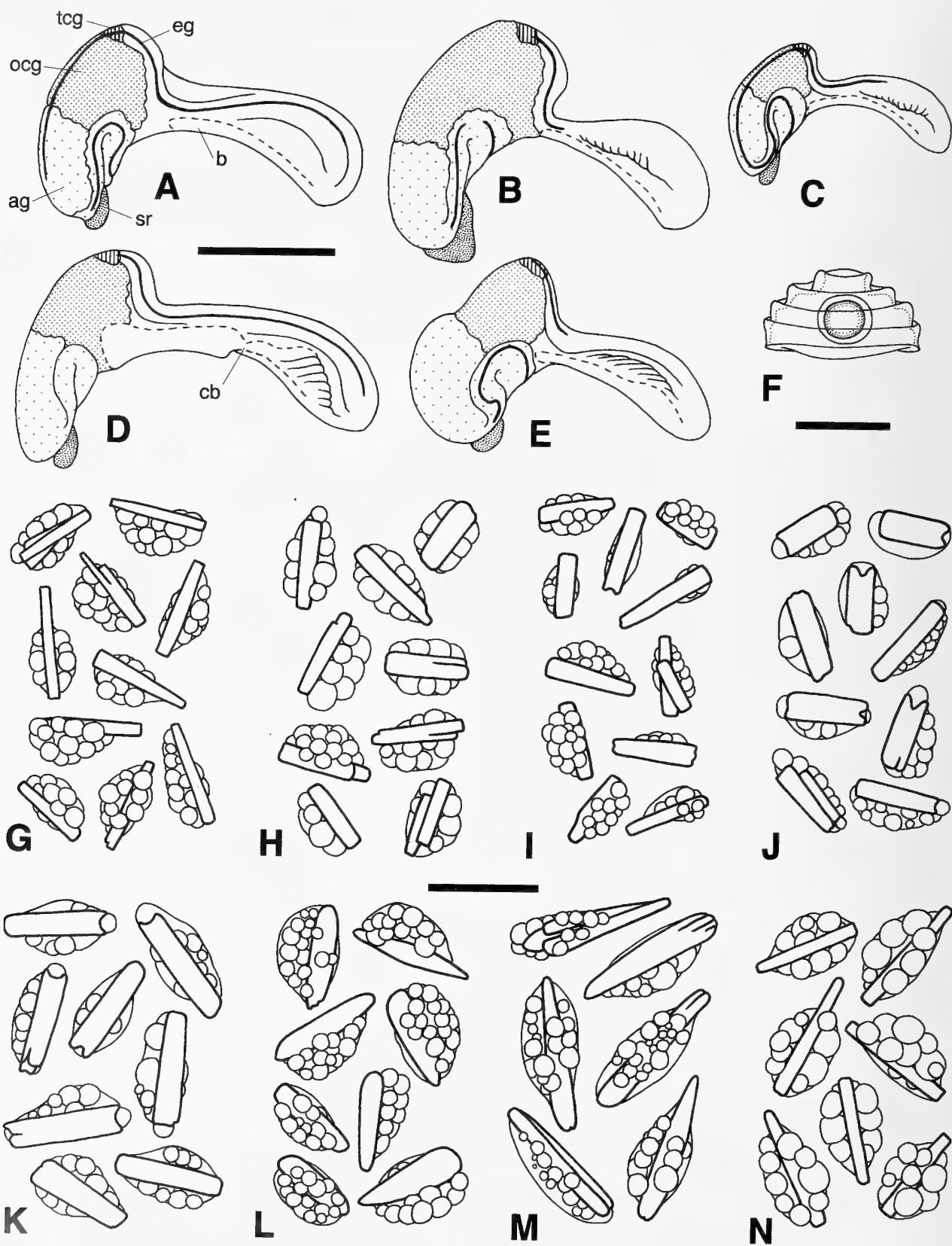
**Etymology:** Name derived from the type locality on the Peninsula Santa Elena, Ecuador.

**Types (Figure 1R):** Holotype BMNH 20000309. 19 paratypes BMNH 20000310 (Figures 1S, T); 100 paratypes in alcohol BMNH 20000311, 4 paratypes USNM 894294. Type locality: Punta Carnero, Peninsula Santa Elena, Guayas Province, Ecuador.

**Taxonomic history:** In ANSP there is a sample of six shells of this species collected by C. E. White in Ecuador, bearing the name *Lacuna guayaquilensis* Bartsch (ANSP 144844); the name was apparently never published by Bartsch. *Fossarus guayaquilensis* Bartsch, 1928, is a different taxon, a true member of the genus *Fossarus* (Bartsch, 1928). In some lists of Peruvian mollusks there appears the name *Littorina umbilicata*, without locality (Dall, 1909; Alamo & Valdivieso, 1987, 1997; Paredes et al., 1999; also world list of Littorinidae in Rosewater, 1970). This is a misidentification (see Taxonomic History of *N. atrata*), but might possibly be intended for the present species (perhaps also including *N. atrata*).

**Diagnosis:** Shell small, narrowly turbanate; smooth with impressed lines, or with low ribs and microstriae; pseudo-umbilicus small; usually imperforate; commonly brown with basal and sutural white band. Penis with slightly pointed, twisted filament tip, small mamilliform gland on short projection of base, no glandular disc.

Figure 3. Penes of *Nodilittorina porcata* group: *N. atrata* (A–G), *N. porcata* (H–O), *N. santelenae* Reid, sp. nov. (P–U), *N. albicarinata* (V, DD–HH), *N. parcipicta* (W–CC), *N. fuscilineata* Reid, sp. nov. (II). A, B. Punta Morales, Golfo de Nicoya, Costa Rica (BMNH 20001159; shell H = 3.9 mm, 3.7 mm). C. Punta Carnero, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20001162; shell H = 2.9 mm). D. Punta Chocolatera, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20001156; shell H = 2.9 mm). E, F. Puerto Ayora, Isla Santa Cruz, Galápagos Islands (BMNH 20001163; shell H = 4.2 mm, 3.5 mm). G. Baltra Channel, Isla Santa Cruz, Galápagos Islands (BMNH 20001160; shell H = 4.5 mm). H–N. Puerto Ayora, Isla Santa Cruz, Galápagos Islands (H, K, BMNH 20001169, shell H = 2.7 mm, 3.1 mm; I, J, BMNH 20001168, shell H = 3.2 mm, 3.3 mm; L, BMNH 20001164, shell H = 3.9 mm; M, N, BMNH 20001167, shell H = 2.8 mm, 3.3 mm). O. Punta Pitt, Isla San Cristobal, Galápagos Islands (BMNH 20001165; shell H = 3.4 mm). P, Q, S, T. Punta Carnero, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20000311; shell H = 3.2 mm, 4.0 mm, S, T, 3.9 mm; two views). R. Anconcito, Guayas, Ecuador (BMNH 20001170; shell H = 2.9 mm). U. Punta Chocolatera, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20001171; shell H = 2.5 mm). V, HH. Playa Coromuel, Baja California Sur, Mexico (V, BNMH 20001186, shell H = 3.4 mm; HH, BMNH 20001185, shell H = 3.6 mm). W. Playa Coromuel, Baja California Sur, Mexico (BNMH 20001174; shell H = 4.0 mm). X, Y. Bahía Santa María, Baja California Sur, Mexico (BMNH 20001173; shell H = 3.8 mm, 4.1 mm). Z, AA, BB. Topolobampo, Sinaloa, Mexico (BMNH 20001176; shell H = 3.7 mm, 3.5 mm; Z, AA, two views). CC. Punta Telmo, Michoacán, Mexico (BMNH 20001181; shell H = 3.6 mm). DD–GG. Balandra, Baja California Sur, Mexico (BMNH 20001182; shell H = 4.7 mm, 5.0 mm; EE, FF, two views, 3.7 mm). II. Punta Carnero, Peninsula Santa Elena, Guayas, Ecuador (BMNH 19990422, holotype of *N. fuscilineata* Reid, sp. nov.; shell H = 2.8 mm). Abbreviations and shading conventions: pg, mamilliform penial gland; ps, penial sperm groove (thick line); r, reservoir of mamilliform penial gland (visible by transparency); sg, subepithelial glandular tissue of penial gland (dotted line; usually visible by transparency); dashed line, cut base of penis; stipple in folds of penial base indicates black pigment. Scale bar = 1 mm.



**Material examined:** 17 lots (including 9 penes, 4 sperm samples, 3 pallial oviducts, 4 radulae).

**Shell (Figures 1R–X):** Mature shell height 1.7–5.6 mm. Shape narrowly to elongately turbinate ( $H/B = 1.13$ – $1.57$ ,  $SH = 1.36$ – $1.84$ ); whorls moderately rounded, slightly angled at periphery, suture distinct. Columella narrow, slightly thickened and convex in center; pseudo-umbilicus usually only a narrow imperforate crescentic area, but sometimes small, perforated. Sculpture variable; smoothest shells have 7–17 impressed striae (more closely spaced posteriorly) above slight rib at periphery of last whorl, 6–9 striae or 2–3 indistinct ribs on base, no additional microstriae, but sculpture sometimes becomes indistinct; sculptured shells show total of 4–12 low rounded or narrow ribs (of which peripheral rib and one to two above only rarely become carinate) on last whorl with spiral microstriae between. Protoconch 2.3 whorls, 0.28 mm diameter. Color variable; darkest shells dark brown to black, with narrow white line or broad band on base; usually with additional white band at suture; base sometimes faintly spotted; paler shells cream with broad grey-brown or indistinctly mottled band between periphery and shoulder; smoothest shells pale yellow-brown with faint brown mottling throughout, and prominent alternating brown and white blotches at shoulder and periphery; aperture brown with anterior (and sometimes posterior) pale stripe, or yellow-brown with external pattern showing through in palest shells; columella purple-brown.

**Animal:** Head black, rarely an unpigmented line across snout, two black lines along tentacle, not meeting at tip; sides of foot speckled black or grey. Opercular ratio 0.38–0.43. Penis (Figures 3P–U): filament tip pointed and twisted, sometimes giving slightly hooked appearance; sperm groove with a kink, distal portion more open, extending to filament tip; single very small mamilliform penial gland on short, narrow projection of base at 0.3–0.5 total penial length; glandular disc absent; base sometimes slightly pigmented. Euspermatozoa 100–107  $\mu\text{m}$ ; paras-

permatozoa (Figures 4J, K) oval; rod-pieces single (rarely two), usually projecting at one or both ends, or at least filling cell, 11–23  $\mu\text{m}$ , oblong, parallel-sided, ends blunt or hollowed; granules large, spherical, distinct. Pallial oviduct (Figure 4C) with flexure and constriction between spiral and straight sections; copulatory bursa opening at anterior end of straight section, extending back to capsule gland. Spawn not observed; protoconch indicates planktotrophic development.

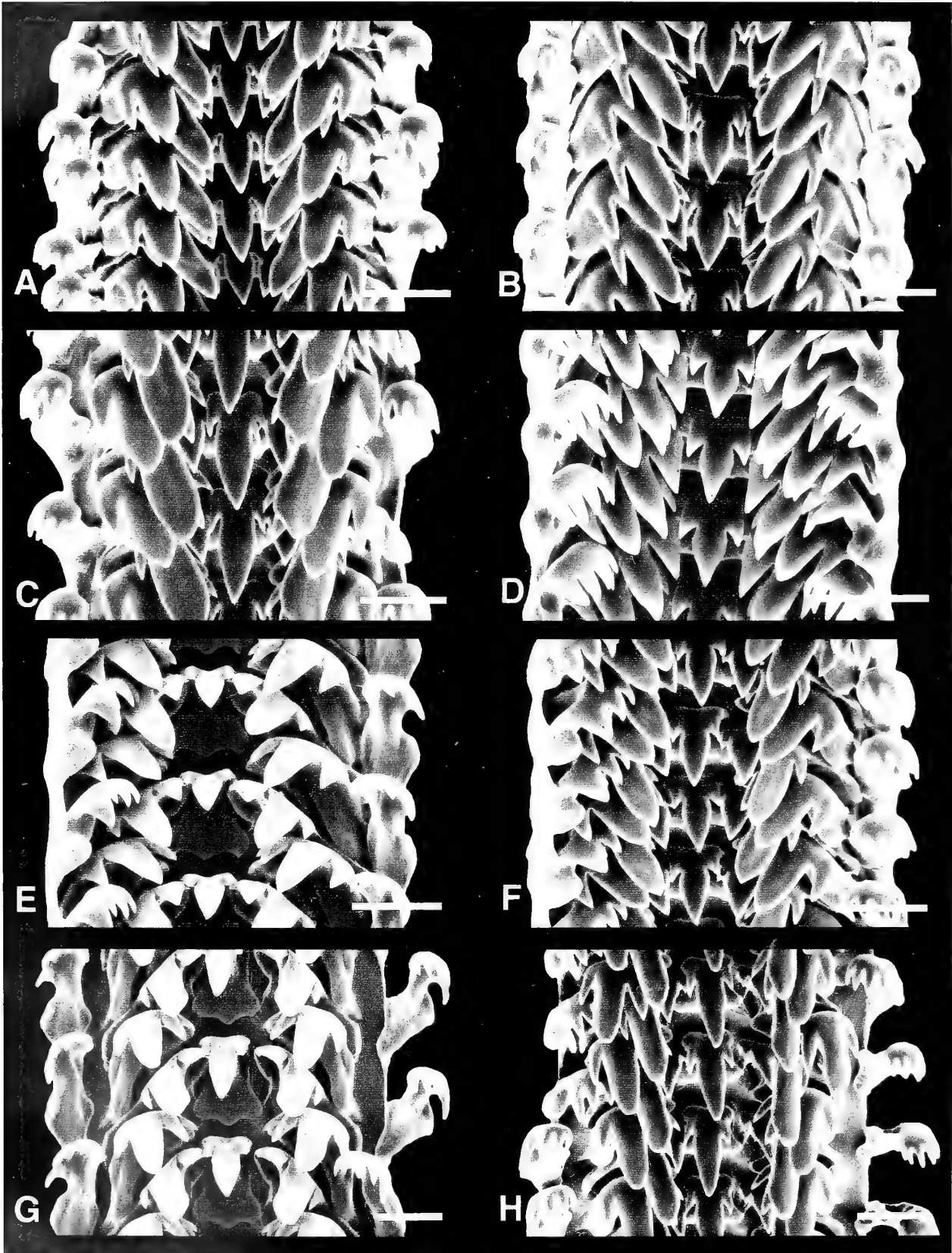
**Radula (Figure 5C):** Relative radular length 1.0–2.1. Rachidian: length/width 1.13–1.77; major cusp pointed and elongate leaf-shaped. Lateral and inner marginal: major cusps pointed or slightly rounded. Outer marginal: six cusps.

**Habitat:** Among barnacles in mid to upper eulittoral, often in empty tests; in shallow pools with fine filamentous green algae, on rock platform, upper eulittoral; on sandstone and mudstone; semi-sheltered bays and wave-exposed headlands; usually abundant.

**Range (Figure 6):** Southern Ecuador and northern Peru. Recorded from Peninsula Santa Elena, Guayas, Ecuador (Punta Carnero, BMNH 20000310; Anconcito, BMNH 20001170; Punta Chocolatera, BMNH 20001171; La Libertad, LACM 66-116; Playas, LACM 70-13); El Rubio and Punta Mero, Tumbes, Peru (LACM 72-85); Talara, Piura, Peru (USNM 368553, 1 specimen); Paita, Piura, Peru (USNM 368579, 368580, 1 specimen each).

**Remarks:** This species has a narrowly restricted range and is therefore seldom represented in museum collections. In the field, it is microsympatric with *N. atrata* among barnacles, but extends lower on the shore, and is much more common than that species where they occur together (personal observation, Peninsula Santa Elena); juvenile *N. paytensis* can also be found commonly in this microhabitat. The habitat among filamentous algae in shallow pools is unusual for this genus, and here it was found abundantly with only very few *N. atrata* (personal

Figure 4. Pallial oviducts (A–E), egg capsule (F) and paraspermatozoa (G–N) of *Nodilittorina porcata* group: *N. atrata* (A, F, G, H), *N. porcata* (B, I), *N. santelenae* Reid, sp. nov. (C, J, K), *N. parcipicta* (D, L, M), *N. albicarinata* (E, N). A, F. Punta Morales, Golfo de Nicoya, Costa Rica (BMNH 20001159; shell H = 5.2 mm). B, I. Puerto Ayora, Isla Santa Cruz, Galápagos Islands (BMNH 20001169; shell H = 5.0 mm). C, K. Punta Carnero, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20000311; shell H = 5.0 mm). D. Bahía Santa María, Baja California Sur, Mexico (BMNH 20001173; shell H = 4.9 mm). E. Balandra, Baja California Sur, Mexico (BMNH 20001182; shell H = 4.8 mm). G. Punta Carnero, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20000311). H. Muisne, Esmeraldas, Ecuador (BMNH 20001161). J. Anconcito, Guayas, Ecuador (BMNH 20001170). L, M. Playa Coromuel, Baja California Sur, Mexico (BMNH 20001175). N. Playa Coromuel, Baja California Sur, Mexico (BMNH 20001185). Abbreviations and shading conventions for pallial oviducts: ag, albumen gland (light stipple; opaque and translucent portions not usually distinguishable in gross dissection); b, copulatory bursa (dashed line; visible by dissection; only the part separated as a sac from lumen of straight section of pallial oviduct is indicated); cb, constriction in copulatory bursa; eg, egg groove (thick line; visible externally when darkly pigmented and if not concealed by swollen oviducal glands); ocg, opaque capsule gland (mid stipple); sr, seminal receptacle (heavy stipple); teg, translucent capsule gland (cross-hatching); some internal folding of lumen in straight section is visible by transparency. Scale bars A–E = 1 mm; F = 0.1 mm; G–N = 20  $\mu\text{m}$ .





observation, Anconcito, Peninsula Santa Elena). As in all members of the *N. porcata* group, sculpture and color vary widely; the palest, smoothest, most elongate shells were found among algae in the sheltered microhabitat of a shallow pool, whereas samples from among barnacles on exposed headlands were dark, ribbed, and lower spired. This distinction was maintained over a distance of a few meters on a shore at Anconcito, suggesting that ecophenotypic influences may be important (see Discussion).

Confusion is possible with two species with which it is syntopic, *N. atrata* and *N. paytensis*. The former has a more rounded, globular, slightly patulous shape, with a distinctive black and white pattern (including a prominently striped base) or entirely white shell; the penis of *N. atrata* has a narrower tip and larger mamilliform penial gland, carried on a longer projection of the base. Juvenile *N. paytensis* of similar size are tall-spired, with flat whorls giving a conical outline, and an angled periphery; the surface is glossy, with regular incised primary grooves and no raised ribs; the color pattern of dark brown with pale sutural and basal bands is similar, but brown axial flames are usually prominent, especially at the suture. *Nodilittorina santelenae* was also syntopic with *N. fuscolineata* at Punta Carnero, the type locality of both; the latter species is more low spired and globular, with a lined or dashed pattern on prominent ribs, and penial form is diagnostic of both. The smooth-shelled form of *N. santelenae* shows a remarkable convergence with the shell of the broadly sympatric (but not syntopic) *Littoraria rosewateri* Reid; although the latter is larger (5–12 mm), coloration and sculpture are similar (cf. Reid, 1999a: fig. 9A), but anatomically it is quite different (the penis lacks a mamilliform gland but shows a glandular disc, the penial valve is closed and the oviduct is multispiral) and the usual habitat is among supralittoral marsh grass in mangrove areas, so confusion is unlikely.

*Nodilittorina fuscolineata* Reid, sp. nov.

(Figures 2A–E, 3II, 5D, 6)

**Etymology:** Latin: “dark-lined,” in reference to color pattern.

**Types:** Holotype BMNH 19990422 (Figure 2A). Type

locality: Punta Carnero, Peninsula Santa Elena, Guayas Province, Ecuador.

**Taxonomic history:** This species has not previously been recognized as distinct. It is rare in museum collections, and generally found in mixed lots with *N. atrata*. Some of the authors writing on *N. atrata* (and its synonyms) in Central America (see Synonymy of *N. atrata*) might have included this species, but owing to its rarity this is unlikely.

**Diagnosis:** Shell small, globular to turbate, often translucent; usually with strong spiral ribs and microstriae; large, perforated pseudo-umbilicus; pale, with brown lines or long dashes on ribs. Penis with long, pointed filament, small mamilliform gland closely attached to base, no glandular disc.

**Material examined:** 15 lots (including 1 penis, 1 radula).

**Shell (Figures 2A–E):** Shell height to 2.8 mm. Shape turbate to globular or slightly patulous ( $H/B = 1.00–1.25$ ,  $SH = 1.38–1.67$ ); whorls well rounded, suture distinct; usually delicate and translucent. Columella straight, narrow, expanded and flattened at base; pseudo-umbilicus moderate to large, perforated, outlined by sharp keel continuous with outer apertural lip. Sculpture of rather uniform spiral ribs; on spire whorls 1–3 ribs visible; on last whorl 2–3 ribs on base, peripheral rib, and 3–4 ribs above periphery, rarely with 1 or 2 smaller interpolated riblets on last whorl; ribs vary from low and rounded to sharp and raised, but are rarely absent; spiral microstriae present over entire surface. Protoconch 3.0 whorls, 0.34 mm diameter, with fine spiral riblets. Color cream to pale brown, usually with continuous brown lines or long dashes on ribs, and a band or a few large blotches at suture; occasionally pattern is irregularly marbled, but sutural blotches and lines on base remain visible; aperture with external pattern showing through, columella purplish or pale brown.

**Animal (description of holotype):** Head black, narrow unpigmented stripe across snout, two black lines along tentacle, not meeting at tip; sides of foot black. Penis (Figure 3II): filament long, pointed, not twisted; sperm groove extending to filament tip; single small mamilliform penial gland closely attached to base at 0.25 total

Figure 5. Radulae of *Nodilittorina porcata* group: *N. atrata* (A), *N. porcata* (B), *N. santelenae* Reid, sp. nov. (C), *N. fuscolineata* Reid, sp. nov. (D), *N. parcipicta* (E, F), and *N. albicarinata* (G, H). A. Puerto Ayora, Isla Santa Cruz, Galápagos Islands (BMNH 20001163; at 45°; shell H = 5.0 mm). B. Puerto Ayora, Isla Santa Cruz, Galápagos Islands (BMNH 20001169; at 45°; shell H = 5.0 mm). C. Punta Carnero, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20000311; at 45°; shell H = 5.0 mm). D. Punta Carnero, Peninsula Santa Elena, Guayas, Ecuador (BMNH 19990422, holotype of *N. fuscolineata* Reid, sp. nov.; at 45°; shell H = 2.8 mm). E, F. Topolobampo, Sinaloa, Mexico (BMNH 20001176; two views of radula, flat and at 45°; shell H = 4.7 mm). G, H. San Felipe, Baja California Norte, Mexico (BMNH 20001183; two views of radula, flat and at 45°; shell H = 5.2 mm). Scale bars = 20  $\mu$ m.



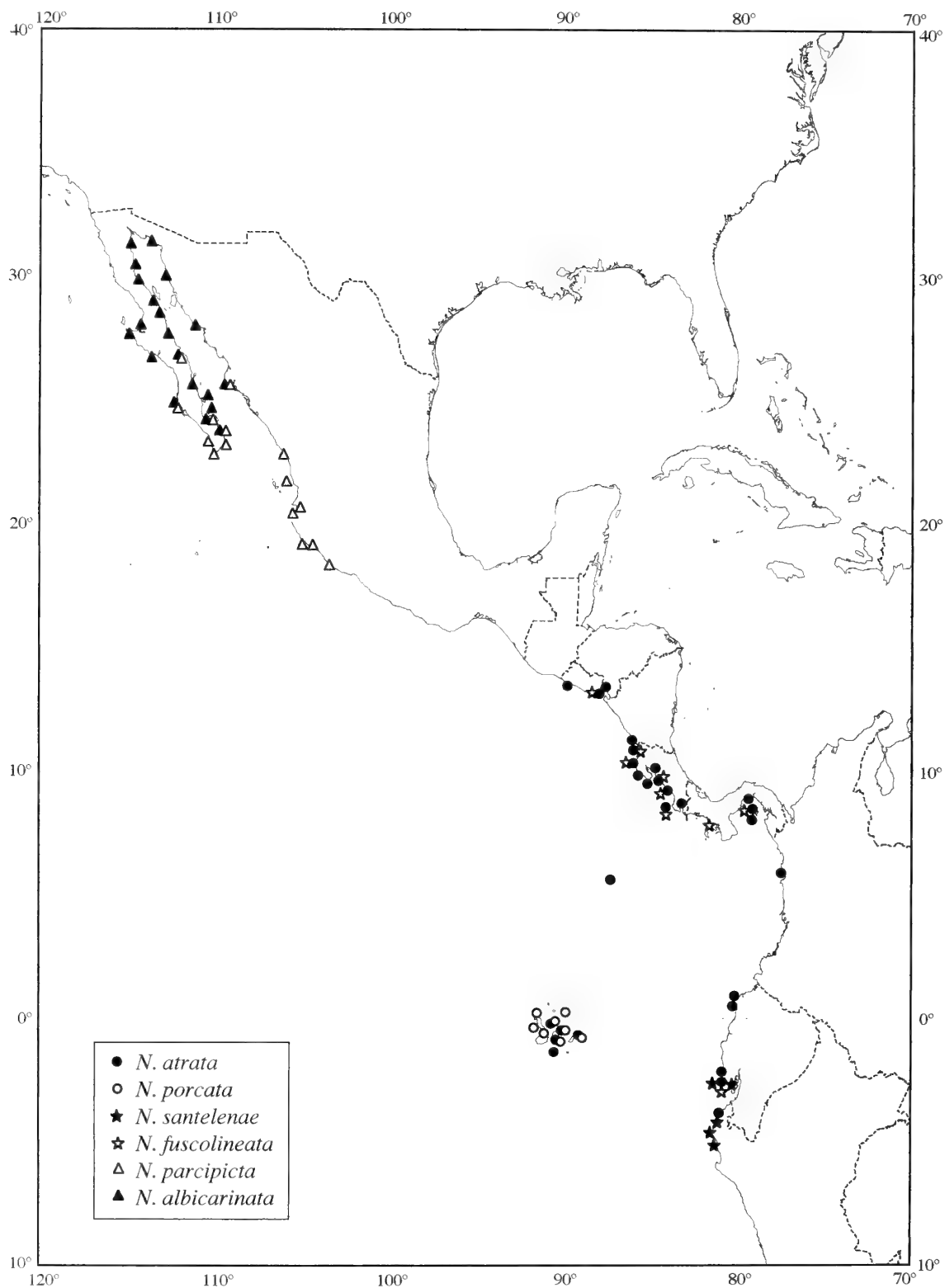


Figure 6. Geographical distribution of *Nodilittorina porcata* group (all records based on material examined).

penial length; glandular disc absent; base slightly pigmented. Sperm not seen. Pallial oviduct not seen. Spawn not observed; protoconch indicates planktotrophic development.

**Radula (Figure 5D):** Relative radular length unknown. Rachidian: length/width 1.06; major cusp pointed and leaf-shaped. Lateral and inner marginal: major cusps moderately pointed. Outer marginal: 6 cusps.

**Habitat:** Of the available collections, only the holotype was collected alive, among barnacles in the upper eulittoral, on an exposed siltstone headland on a sandy coast. The typical habitat of this species is uncertain. All other material seen (14 lots in LACM) was collected dead in sediments from depths of 2–100 m, often mixed with *N. atrata*; neither *N. atrata* nor any other member of the genus occurs subtidally, so this must represent material washed from the eulittoral zone. Many live collected samples of the broadly sympatric *N. atrata* are available from within the range of this species, yet only at the type locality has a single *N. fuscilineata* been found among them. A possible explanation is that this species is found in relatively exposed or offshore localities (where sampling is less frequent), unlike *N. atrata* which occupies a range of habitats. This is supported by the fact that many of the localities for dead *N. fuscilineata* (as well as the type locality) are peninsulas or islands; furthermore, it appears to be absent from relatively sheltered mainland sites such as the Panama Canal Zone and the Golfo de Nicoya in Costa Rica (both represented by numerous and large collections of *N. atrata*).

**Range (Figure 6):** El Salvador to southern Ecuador. Range limits: Isla Zacatillo, Golfo de Fonseca, El Salvador (LACM 73-57, 1 specimen); Bahía Jobo, Costa Rica (LACM 72-17); Isla del Cano, Costa Rica (LACM 72-63); Bahía Honda, Panama (LACM 38-131, 1 specimen); Isla Taboga, Panama (LACM 65-25, 1 specimen); Punta Carnero, Peninsula Santa Elena, Guayas, Ecuador (BMNH 19990422, holotype only).

**Remarks:** As mentioned above, this species is so far represented by only a single live-collected specimen, as well as 50 dead shells from 14 localities (LACM), and is therefore by far the rarest of the *N. porcata* group in museums. This may reflect a less accessible habitat (see Habitat).

The shell of *N. fuscilineata* is distinctive, with regular ribs marked by brown lines or dashes, well rounded whorls and delicate texture, and these characters make it easily separable from the broadly sympatric *N. atrata* (a larger, more solid shell, very variable in form, but never with such rounded spire whorls nor with a lined pattern). The shell characters are, however, somewhat similar to those of *N. parcipicta*, although that species is larger (to 5.9 mm), more solid, and has a finer spotted (rarely dashed, and never lined) pattern on the ribs, or may even be unpatterned. Fortunately, the sole living specimen ex-

amined was a male and, assuming this to be typical, the penial shape is diagnostically different from those of both *N. atrata* and *N. parcipicta*. The penis is more similar to that of the latter in its lack of a marked twist to the filament tip. Their geographical ranges are not known to overlap, *N. parcipicta* being restricted to Mexico. These shell and penial similarities suggest that *N. fuscilineata* and *N. parcipicta* are sister species. If the inferred habitat of *N. fuscilineata* on exposed shores is correct, this is another similarity between the two.

### *Nodilittorina parcipicta* (Carpenter, 1864)

(Figures 2F–M, 3W–CC, 4D, L, M, 5E, F, 6, 22F)

*Fossarus parcipictus* Carpenter, 1864a:476 (Cape St Lucas [Cabo San Lucas, Baja California, Mexico]; lectotype (Palmer, 1963) USNM 4060, seen, Palmer, 1963:pl. 65, figs. 4, 5, Figure 2L herein; paralectotype USNM 678706, seen). Palmer, 1963:342, pl. 65, figs. 4, 5. Keen, 1971:454. Abbott, 1974:136 (may include *N. atrata* or *N. fuscilineata*). Skoglund, 1992:34.

*Fossarus* cf. *atratus*—McLean, 1970:127 (not *Littorina atrata* C. B. Adams, 1852 = *N. atrata*).

**Taxonomic history:** This species has been largely neglected since its description, and has not hitherto been referred to a littorinid genus. The lectotype was figured by Palmer (1963), and the name was listed by Keen (1971) and Abbott (1974). The latter gave a range “Baja California to Panama,” but the species does not occur so far south, and records of *N. atrata* and/or *N. fuscilineata* may have been included. In describing the new species *N. albicarinata*, McLean (1970) remarked that it often occurred together with “a species of *Fossarus*, cf. *atratus*” by which, from the brief description, he apparently intended *N. parcipicta*; this is supported by the inclusion of one specimen of *N. parcipicta* among the paratypes of *N. albicarinata* (LACM 1400).

**Diagnosis:** Shell small, globular to turbinate; strong spiral ribs and microstriae; large, perforated pseudo-umbilicus; white or yellow, with small brown spots on ribs. Penis with broad, blunt filament, large mamilliform gland closely attached to base, no glandular disc.

**Material examined:** 30 lots (including 15 penes, 4 sperm samples, 5 pallial oviducts, 4 radulae).

**Shell (Figures 2F–M, 22F):** Mature shell height 2.3–5.9 mm. Shape turbinate to globular or slightly patulous ( $H/B = 0.83\text{--}1.25$ ,  $SH = 1.27\text{--}1.74$ ); whorls rounded, suture distinct. Columella straight, narrow, expanded, and flattened at base; pseudo-umbilicus usually large, perforated, outlined by sharp keel continuous with outer apertural lip, but sometimes narrow or imperforate. Sculpture of rather uniform, sharp spiral ribs; on spire whorls only 1–2 ribs visible; on last whorl 2–3 ribs on base, peripheral rib, and 2–4 ribs above periphery, but with smaller interpolated ribs on largest shells total number on

last whorl up to 11; ribs vary from low to strongly carinate; spiral microstriae present over entire surface (Figure 22F). Protoconch 2.7 whorls, 0.34 mm diameter. Color white to pale yellow, sometimes lacking pattern, but usually with small brown spots on ribs; darkest shells have irregular axial stripes, blotches or transverse dashes of dark brown or black; aperture with external pattern showing through, only rarely with peripheral dark band or pale anterior stripe, columella cream to dark brown.

**Animal:** Head black to grey, unpigmented stripe across snout, two black lines along tentacle, not meeting at tip; sides of foot speckled black to pale grey. Opercular ratio 0.39–0.42. Penis (Figures 3W–CC): filament broad, blunt, or minutely pointed, not (or only slightly) twisted; sperm groove extending to filament tip; single large mamilliform penial gland closely attached to base at 0.2–0.4 total penial length (mamilliform gland absent in one specimen); glandular disc absent; base often slightly pigmented. Euspermatozoa 64–93  $\mu\text{m}$ ; paraspermatozoa (Figures 4L, M) oval; rod-pieces single (rarely 2–3), usually projecting at both ends or at least filling cell, 16–28  $\mu\text{m}$ , slightly tapering or fusiform, ends rounded; granules large, spherical, distinct. Pallial oviduct (Figure 4D) with flexure and constriction between spiral and straight sections; copulatory bursa opening near anterior end of straight section, constricted at about one-third of its length, extending back beneath capsule gland. Spawn not observed; protoconch indicates planktotrophic development.

**Radula (Figures 5E, F):** Relative radular length 1.2–2.1. Rachidian: length/width 1.06–1.44; major cusp pointed and elongate leaf-shaped. Lateral and inner marginal: major cusps pointed or slightly rounded. Outer marginal: 5–6 cusps.

**Habitat:** In small, shallow rock pools in upper eulittoral; in crevices among barnacles; on granite, conglomerate and concrete; wave-exposed headlands, and sometimes sheltered bays.

**Range (Figure 6):** Southern Baja California, mainland coast of Mexico from Sinaloa to Michoacán. Range limits: Bahía Magdalena, Baja California Sur (USNM 218336, 1 specimen); Punta Lobos, Todos Santos, Baja California Sur (BMNH 20001172); Bahía Santa Maria, near Cabo San Lucas, Baja California Sur (BMNH 20001173); Playa Coromuel, 3 km N of La Paz, Baja California Sur (BMNH 20001175); El Requeson and El Coyote, Bahía Concepción, Baja California Sur (LACM 63-37); Topolobampo, Sinaloa (BMNH 20001176); Mazatlán, Sinaloa (BMNH 20001177); Punta Telmo, Michoacán (BMNH 20001181). This species only just penetrates the Gulf of California, but is common at its limits at both Bahía Concepción and Topolobampo. The lack of records south to Oaxaca may simply reflect lack of collecting of this small species with a preference for exposed and inaccessible localities.

**Remarks:** This species is somewhat less variable in shell characters than others in the *N. porcata* group; most specimens show a spotted pattern, and it is not known to occur in a smooth form. Although sometimes found on sheltered shores, most of the available samples are from wave-exposed shores, which are more strongly exposed than shores on which other members of this group have been found. This, combined with its restriction to the shores of Baja California and southern Mexico, and absence from most of the Gulf of California, emphasizes the oceanic character of its distribution. A similar distribution on the coast of Mexico is shown by the likewise oceanic species *Littoraria pintado pullata* (Carpenter), although that species also occurs on the eastern Pacific islands (Reid, 1999a).

There is limited sympatry between *N. parcipicta* and *N. albicarinata*, but the latter is found only in moderately sheltered habitats, and there are only three recorded instances of syntopy. At Playa Coromuel (personal observation) both species were common among the uppermost barnacles on a sheltered shore, but *N. parcipicta* was found mainly at slightly lower tidal levels than *N. albicarinata*, and the spire whorls of the former were more strongly eroded. At Topolobampo (personal observation) only a single *N. albicarinata* was found together with moderately common *N. parcipicta*, among sparse barnacles on a concrete ramp, in a sheltered, silty bay. In addition, both species are recorded from El Requeson, Bahía Concepción (LACM 1400), and said to occur together by McLean (1970). These two species are easily distinguished by shell characters; *N. albicarinata* is usually imperforate, often somewhat tall-spined, smooth, and grey to white in color, unlike any shell of *N. parcipicta*; sculptured forms of *N. albicarinata* are sharply carinate at the shoulder and periphery, and (at least on the spire whorls) the carinae are white on a brown ground, unlike the more globular, umbilicate shells of *N. parcipicta* with regular spotting on uniform, rounded ribs.

A more similar shell is that of *N. fuscolineata*, but that is distinguished by smaller size, delicate texture, and pattern of brown lines or long dashes on the ribs; the two are allopatric (*N. fuscolineata* occurring to the south of El Salvador) and are possible sister species (see Remarks on *N. fuscolineata*).

#### *Nodilittorina albicarinata* (McLean, 1970)

(Figures 2N–W, 3V, DD–HH, 4E, N, 5G, H, 6)

*Littorina dubiosa*—McLean, 1961:464 (not C. B. Adams, 1852 = *N. dubiosa*).

*Littorina albicarinata* McLean, 1970:127, fig. 36 (El Requeson, Concepcion Bay, Baja California, 26°38'N, 111°50'W; holotype LACM 1399, seen, Figure 2O; 263 paratypes LACM 1400, seen, one is *N. parcipicta*; 4 paratypes USNM 681630, seen; 4 paratypes each AMNH, ANSP, CAS, MCZ, SBM). Keen, 1971:365, fig. 180.

*Littorina (Littorinopsis) albicarinata*—Abbott, 1974:69, fig. 566.

*Littorina (Fossarilittorina) albicarinata*—Rosewater, 1981:30.

*Nodilittorina (Nodilittorina) albicarinata*—Reid, 1989a:99. Skoglund, 1992:15.

**Taxonomic history:** McLean (1961) at first identified the smooth form of this species as *Littorina dubiosa*, and suggested that keeled shells might belong to the same species. Later (1970), he described the distinctive form with white carinae under the new name, and again remarked on the variation in sculpture. The species is abundant within its range, and the paucity of literature references is a reflection of the relatively few malacological studies in the Gulf of California. In ANSP and USNM there are several lots of the tall-spined, smooth, white form of this species labelled "*Littorina cognatus* Hemphill, MS"; this name does not appear to have been published and was not included in the list of Hemphill's taxa by Coan & Roth (1987).

**Diagnosis:** Shell small, turbate to tall; smooth with impressed striae, or carinate with microstriae; narrow, imperforate pseudo-umbilical area; often grey to white; if patterned, then white carinae on brown shell, especially on spire. Penis with pointed, slightly twisted filament tip, opening of sperm groove behind tip, large mamilliform gland on stout or long lateral appendage, no glandular disc.

**Material examined:** 40 lots (including 19 penes, 4 sperm samples, 6 pallial oviducts, 7 radulae).

**Shell (Figures 2N–W):** Mature shell height 2.3–7.6 mm. Shape turbate to tall-spined ( $H/B = 1.13–1.77$ ;  $SH = 1.46–2.30$ ); spire whorls rounded, suture distinct; periphery of last whorl only slightly angular, but may be marked by a rib or carina; solid. Columella straight, narrow, slightly pinched at base of pillar; pseudo-umbilicus usually only a narrow, imperforate area or absent, but sometimes narrowly perforated. Sculpture variable; smoothest shells with 6–13 impressed lines above periphery (sometimes increasing to 23 on last whorl) and similar but less distinct fine striae on base, periphery usually marked by a slight rib; almost all shells have a prominent rib at shoulder and another at periphery of early spire whorls (may be lost by erosion), even if they become smooth on last whorl; strongly sculptured shells with shoulder and peripheral ribs persisting as sharp carinae, with additional 2 ribs below suture, 1 between carinae, and 3 on base, giving total of 8 more or less carinate ribs, with coarse microstriae between; periostracum occasionally produced into minute bristles (less than 100  $\mu\text{m}$ ) on basal and peripheral ribs of strongly sculptured shells. Protoconch 2.8 whorls, 0.29–0.34 mm diameter. Color variable; spire usually brownish with white dashes or lines marking shoulder and peripheral rib; pattern may persist, with white carinae and ribs on brown ground; shells often

white, fawn, or chalky blue-grey on last whorl; occasionally with fine brown spots, mottling or fine axial zigzags on last whorl, strongest at suture and periphery; color pattern always fades to white toward inner part of base; columella and aperture brown, with anterior unpigmented band, columella sometimes white.

**Animal:** Head and sides of foot black; two black lines along tentacle, meeting (or almost so) at small black terminal spot. Opercular ratio 0.44–0.51. Penis (Figures 3V, DD–HH): filament tip pointed and slightly pinched; sperm groove with a kink, not extending to filament tip; single large mamilliform penial gland on stout (and in fully relaxed specimens very long) lateral appendage at 0.4–0.5 total penial length; glandular disc absent (but in contracted specimens an extension of glandular material of mamilliform gland may resemble a small glandular disc); base pigmented. Euspermatozoa 71–86  $\mu\text{m}$ ; paraspermatozoa (Figure 4N) oval; rod-pieces single, filling cell or projecting at one or both ends, 16–24  $\mu\text{m}$ , blunt or slightly rounded at ends, parallel-sided or occasionally slightly tapering; granules large, spherical, distinct. Pallial oviduct (Figure 4E) with flexure and constriction between spiral and straight sections; copulatory bursa opening at anterior end of straight section, extending back to start of spiral portion. Spawn not observed; protoconch indicates planktotrophic development.

**Radula (Figures 5G, H):** Relative radular length 1.9–3.1. Rachidian: length/width 1.13–1.54; major cusp pointed and elongate leaf-shaped. Lateral and inner marginal: major cusps pointed or slightly rounded. Outer marginal: 6–8 cusps.

**Habitat:** Among uppermost barnacles and in crevices, upper eulittoral; on volcanic conglomerate, basalt, concrete; sheltered coasts; often abundant; habitat notes with one lot (Puerto Lobos, Sonora, Mexico, USNM 862206) "in grasses," presumably supralittoral halophytic salt-marsh grass.

**Range (Figure 6):** Southwestern Baja California and Gulf of California. Range limits: Laguna Manuel, Baja California Norte (USNM 106528); Punta Abreojos, Baja California Sur (USNM 265774); Bahía Magdalena, Baja California Sur (USNM 332443); Ensenada de los Muertos, Baja California Sur (G. J. Vermeij Collection); Balandra, 30 km N of La Paz, Baja California Sur (BMNH 2001182); San Felipe, Baja California Norte (BMNH 20001183); Puerto Peñasco, Sonora (USNM 665246); Punta San Antonio, Guaymas, Sonora (LACM 73-6); Topolobampo, Sinaloa (BMNH 2001184, 1 specimen). The distribution of this species is apparently disjunct, with no records from the inhospitable exposed coast between La Paz and Bahía Magdalena. It is apparently common farther north in suitable lagoonal and sheltered habitats on the western coast of Baja California. Although common

Table 2

Summary of the most useful characters for the identification of the two species of the *Nodilittorina modesta* group.

Character	<i>N. modesta</i>	<i>N. conspersa</i>
1. Geographical range	Baja California, Mexico, Costa Rica, Clipperton Atoll	Oaxaca (Mexico), El Salvador to Ecuador, Isla del Coco, Galápagos Islands
2. Shell		
—primary grooves on spire whorls	usually 6–7	usually 4–5
—all white form	yes, especially in strongly sculptured shells	no, always patterned
—color pattern	if present, minute grey-brown dots on white shell, often becoming obsolete on last whorl	always with small orange-brown dots on white shell
3. Tentacle pattern	fine transverse black lines	two longitudinal black lines
4. Penis		
—filament length	long, 0.7–0.8 total length	short, 0.25–0.35 total length
—filament tip	tapering to pointed tip	bluntly hooked tip

at Guaymas, only a single specimen was found in a suitable habitat at Topolobampo (personal observation).

**Remarks:** Confusion is possible with two or three sympatric species. It is only rarely syntopic with *N. parvipicta* among barnacles, in the narrow zone of sympatry in southern Baja California and the southeastern Gulf of California, but the shells of these two species are readily discriminated (see Remarks on *N. parvipicta*). In the Gulf of California *N. albicarinata* occurs on the same shores as *N. penicillata*, a larger species lacking carinate sculpture, of which juveniles show a diagnostic shell pattern of axial brown lines with a spiral brown line on the shoulder and another on the base. Another broadly sympatric species is *Littoraria rosewateri* Reid; although there are no available records of these two occurring syntopically, they may well do so, since the typical habitat of *L. rosewateri* is among supralittoral marsh grasses, from which a sample of *N. albicarinata* has been collected (see Habitat). The shells of these two species can be remarkably similar. *Littoraria rosewateri* closely resembles tall-spired, smooth forms of *N. albicarinata*, but the former reaches larger size (5–12 mm), never shows an enlarged peripheral or shoulder rib on early whorls or on last whorl, the color is polymorphic in large samples, and there is never a pale anterior band within the aperture. Anatomical characters are diagnostic; the tentacles of *L. rosewateri* show transverse bands, the penis has a large glandular disc and no mamilliform penial gland, and the pallial oviduct is multispiral. It is interesting that the algal-dwelling form of *N. santelenae* also shows convergence with *L. rosewateri*.

Recently it has been suggested that the radulae of *Littoraria* species show ecophenotypic plasticity of cusp shape according to the substrate, whether rock or plants (Reid & Mak, 1999). It is unusual to find a member of the genus *Nodilittorina* on a plant substrate, providing an

opportunity to test this hypothesis in the genus. Two radulae were examined from the sample collected on grasses, but did not display any differences from the rest.

#### The *Nodilittorina modesta* Group

In the older literature, the familiar white littorinids of the eastern Pacific, usually with a pattern of minute brown dots, were generally known by the specific name of *conspersa* Philippi, 1847, but then for the past 30 years by the earlier name of *modesta* Philippi, 1846 (Rosewater, 1970; Keen, 1971; Reid, 1989a). However, close examination of penial shape has revealed two species in this group, with sympatry at two localities in southern Mexico and Costa Rica. The penial differences are small, but entirely consistent even in sympatry, and are correlated with shell differences (Table 2). Philippi (1846a, 1847) named four species in this group, and from his precise descriptions of shell sculpture it is possible to identify the two valid species as *N. modesta* and *N. conspersa*, although their synonymies are complex.

These two species are evidently sister taxa. They share a similar white shell, often with a pattern of brown dots. This pattern is difficult to quantify, since although the dots are laid down along the prosocline growing edge of the shell, their alignment is chiefly in opisthocline series or somewhat irregular, and bears no constant relation to the conspicuous spiral sculpture of the shell. (For comparative purposes, in the descriptions below the dots have been counted along an opisthocline series between periphery and suture on the last whorl). In both species the shell varies from rather smooth to strongly sculptured with spiral grooves, sometimes within a sample, but no correlation with microhabitat or geographical range has been noticed. They share several unusual (probably synapomorphic) anatomical features of the penis, oviduct, and radula. The penis is elongate, lacks a mamilliform

gland, and the glandular base is not clearly differentiated into a glandular disc, in contrast to all other known *Nodilittorina* species except the two Atlantic species *N. meleagris* and *N. mespillum* (Mühlfeld). In the pallial oviduct, a loop of the renal oviduct projects into the center of the spiral loop of the albumen gland, which has not been seen in other *Nodilittorina* species. The radulae share the presence of an additional pair of denticles at the concave anterior edge of the rachidian tooth, which is absent elsewhere in the genus (or only slightly and variably developed in some individuals of the *N. aspera* group). Their habitat is also similar, in the upper eulittoral on wave-exposed shores, whereas other large species of the genus occupy the littoral fringe.

Specimens of both species from localities in Oaxaca and Costa Rica sometimes contained a commensal polychaete platyhelminth (R. Sluys, personal communication). Single worms up to 5.5 mm long are found in the mantle cavity, which they may fill completely, although the host sustains no apparent damage. A similar commensal has been observed in *N. apicina* and *N. tenuistriata*, but has not apparently been recorded in other littorinids.

### *Nodilittorina modesta* (Philippi, 1846)

(Figures 7A–F, 8A–F, N, P, Q, 9A, B, 10, 22G, H)

*Littorina modesta* Philippi, 1846a:141 (Sitka, Nova Albion [Alaska]; insulam Mauriti [Mauritius]; both in error, here restricted to Mazatlán, Mexico; lectotype (here designated, 19.6 × 13.2 mm, 'Sitka') BMNH 1968224, seen, Philippi, 1847:*Littorina* pl. 6, fig. 12, Figure 7E herein; 3 paralectotypes BMNH 1968224, seen; 3 additional specimens probably from same lot, BMNH 19990404, seen). Middendorff, 1849:394. Carpenter, 1857a:216, 224, 286. Reeve, 1858:sp. 107, pl. 18, fig. 107. Keen, 1958:282. Keen, 1971:366, fig. 183 (in part, includes *N. conspersa*). Holguín & González, 1989:115, fig.

*Littorina modesta*—Philippi, 1847:3:48–49, *Littorina* pl. 6, fig. 12. Menke, 1851:164. Carpenter, 1857a:237, 326. Weinkauff, 1882:52–53, pl. 6, figs. 13, 16.

*Littorina sitchana* var. *modesta*—Carpenter, 1864b:655.

*Littorina (Littorinopsis) modesta*—Rosewater, 1970:423. Abbott, 1974:69 (in part, includes *N. conspersa*).

*Nodilittorina* (? *Fossarilittorina*) *modesta*—Reid, 1989a:98 (in part, includes *N. conspersa*).

*Nodilittorina modesta*—Emerson, 1995:13 (in part, includes *N. conspersa*).

? *Littorina albida* Philippi, 1848:3:63–64, *Littorina* pl. 7, fig. 9 (Real Llejos [El Realejo, Nicaragua]; types not found). ? Weinkauff, 1882:81, pl. 11, fig. 1.

? *Littorina (Littorinopsis) albida*—von Martens, 1900:576, 582.

*Littorina conspersa*—Carpenter, 1857a:257 (in part, includes *N. conspersa*). Carpenter, 1864b:541, 598, 623 (in part, includes *N. conspersa*). Weinkauff, 1883:217 (in part, includes *N. conspersa*).

*Littorina (Melaraphe) conspersa*—Carpenter, 1857b:346–347 (in part, includes *N. conspersa*).

*Littorina (Melaraphe) aspera* var. *conspersa*—Tryon, 1887:249, pl. 44, figs. 80, 81 (in part, includes *N. conspersa*).

*Littorina (Melarhaphe) conspersa*—von Martens, 1900:577, 586 (in part, includes *N. conspersa*).

*Littorina conspersa*—Pilsbry & Lowe, 1932:124 (not Philippi, 1846). Keen, 1958:282, fig. 174 (in part, includes *N. conspersa*). Villamar, 1965:123 (not Philippi, 1846).

*Littorina (Melarhaphe) philippii* var. *alba* von Martens, 1900:577, 585, pl. 43, fig. 13 (Mazatlan, Mexico; 5 syntypes MNB 102847, seen).

*Littorina (Melarhaphe) conspersa* var. *puncticulata*—von Martens, 1900:577, 786–587 (in part, includes *N. conspersa*; not Philippi, 1847 = *N. conspersa*).

*Littorina aspera*—Keen, 1971:365, fig. 181 (part) (in part, includes *N. aspera*, *N. penicillata*, *N. dubiosa*, *N. apicina*, *N. interrupta*).

**Taxonomic history:** Philippi was meticulous in his descriptions of details of shell shape and sculpture, but he had limited material available, leading him to describe four species in the *N. modesta* group. The identity of the oldest of these, *Littorina modesta* Philippi, 1846, is clear; it was described from material in the Cuming Collection (BMNH), and the specimen figured by Philippi (1847) is here designated lectotype. Philippi (1847) himself described the characteristically numerous and deep grooves of the shell. The listing of *Littorina albida* in the synonymy of *N. modesta* is not certain; the types are lost, and its inclusion is based on the rounded whorls, 5–6 primary spiral grooves, white columella with brown margin, and dark brown posterior aperture, all described by Philippi (1848); this species has not since been recorded from Nicaragua, but it is within the known range. The second species in the *N. modesta* group is here identified as *N. conspersa* (Philippi, 1847), with *Littorina puncticulata* Philippi, 1847, as a synonym. The only other available name was introduced by von Martens (1900) for young specimens of *N. modesta*; these he described as var. *alba* of *Littorina philippii* (itself a synonym of *N. apicina*), apparently misled by the axial lines of brown dots.

Although few subsequent authors have described shells in such detail as Philippi, the largely separate geographical distributions of the two species make it possible to compile the synonymies of each. However, it was an error in the type locality of *Littorina modesta* that led to an initial confusion in the nomenclature of this group. Philippi (1846a, 1847) described *Littorina modesta* from Sitka (Alaska) and Mauritius. Subsequent authors therefore generally used either the names *conspersa* and *puncticulata* (Adams, 1852a, b) or *conspersa* alone (e.g., Carpenter, 1857a, b, 1863, 1864b; Weinkauff, 1883; von Martens, 1900; Keen, 1958) for the two species now recognized in the tropical eastern Pacific. The earlier name *modesta* was correctly used only by Menke (1851); others employed it for a species believed to occur in the northern Pacific (Middendorff, 1849; Carpenter, 1857a, b; Reeve, 1858; Weinkauff, 1882; Keen, 1958). Carpenter (1863, 1864b) even suggested that this enigmatic *modesta* was

a variety of the northern Pacific *Littornia sitkana* Philippi, 1846. This confusion was eventually resolved by examination of the types of *Littorina modesta*, and the name was reinstated for the supposed single species of the tropical eastern Pacific (Rosewater, 1970; Keen, 1971); it has been used in this sense by all subsequent workers.

Some authors have had an even broader concept of these species, combining them in various ways with members of the *N. aspera* group. Tryon (1887) reduced the *N. modesta* group to a subspecies of *Littorina aspera* (followed only by Schwengel, 1938). Von Martens (1900) described heavily marked specimens of *N. modesta* as a variety of *Littorina philippii* (= *N. apicina*), and his figure was reproduced as "*Littorina aspera*" by Keen (1971). It has also been suggested that *N. paytensis* is a southern subspecies of *Littorina modesta* (Keen, 1971; Vermeij, 1973; Rosewater, in Finet, 1985).

Only Philippi (1847), Weinkauff (1882; who followed Philippi's species concepts almost exactly), and C. B. Adams (1852a, b) have previously recognized more than one species in the *N. modesta* group in Central America, basing distinctions on differences in shell outline and degree of sculpture. These features are in fact more variable within the two species of the group than was recognized by these early authors, so that their distinctions do not correspond exactly to that made here on anatomical grounds (see also Taxonomic History of *N. conspersa*).

**Diagnosis:** Shell moderately large, spire whorls moderately rounded; 6–7 primary spiral grooves; sculpture of incised lines only, or with deep grooves 1–3 times rib width; all white or with minute grey-brown dots. Tentacles with fine transverse black lines. Penis with long, tapering filament, glandular flange at base, no mamilliform gland.

**Material examined:** 58 lots (including 26 penes, 4 sperm samples, 7 pallial oviducts, 5 radulae).

**Shell (Figures 7A–F, 22G, H):** Mature shell height 4.5–19.6 mm. Shape high turbinate ( $H/B = 1.33$ – $1.79$ ,  $SH = 1.38$ – $1.89$ ); spire whorls moderately rounded, suture distinct; periphery of last whorl weakly angled. Columella straight, broad, hollowed, and pinched (sometimes with slight protruberance) at base; occasionally a small imperforate pseudo-umbilical area; eroded parietal area in larger shells. Sculpture of 6–7 (rarely 5 or 8) primary spiral grooves on spire whorls; these may remain as incised lines only (1–2 secondary grooves may appear near suture on last whorl), numbering 9–13 above periphery of last whorl (11–17 in total including basal grooves), or become wider and deeper near periphery, but sometimes become obsolete on last whorl; in strongly sculptured shells grooves are wider and deeper throughout, separating rounded ribs on spire whorls, on last whorl, ribs are then raised (occasionally sharp), separated by grooves 1 to 3 times rib width, with narrow interpolated rib ap-

pearing in grooves near suture (rarely in all grooves above periphery), grooves on last whorl then up to 20 above peripheral rib (up to 27 in total); spiral microstriae absent (Figure 22H). Protoconch 2.7 whorls, 0.31 mm diameter, sculptured by spiral ribs (Figure 22G). Color white, with pale brown or lilac-grey apex; often unpatterned (especially when strongly sculptured); otherwise covered with small grey, brown, or black spots aligned in oblique (opisthocline) series (numbering 14–25 spots from peripheral rib to suture on last whorl); spots frequently become obsolete on last whorl; aperture pale orange-brown to dark brown, often darkest posteriorly, with broad pale basal band, usually also a more diffuse shoulder band, external spotting showing through near margin (spotting may be visible even in externally unpatterned shells); columella white to brown, or white pillar with brown margin.

**Animal: Head (Figures 8A, E):** Black to grey, unpigmented stripe across snout, tentacle with fine transverse lines of black or grey, pale beneath; sides of foot black to pale grey. Opercular ratio 0.30–0.38. Penis (Figures 8A–F): filament long (about 0.7–0.8 total length), tapering to pointed or slightly mucronate tip, subepithelial glandular tissue near tip and surrounding sperm groove along anterior edge, filament differentiated from base by smooth anterior edge and slight constriction below swollen glandular sides of sperm groove (differentiation sometimes indistinct); sperm groove open to tip; base with annular wrinkles except at posterior edge with slight glandular flange (opaque subepithelial glandular tissue sometimes visible, approaching surface at minute papilla, although this is not a true mamilliform gland), base occasionally slightly pigmented. Euspermatozoa 57–61  $\mu\text{m}$ ; paraspermatozoa (Figures 8P, Q) round to oval; rod-pieces single (rarely two), filling cell, 13–21  $\mu\text{m}$ , broad, bluntly rounded, hexagonal in section; granules large, spherical, distinct. Pallial oviduct (Figure 8N) with long straight section; large copulatory bursa opening near posterior end of straight section, extending back to albumen gland; small loop of renal oviduct usually projects into center of spiral of albumen gland. Spawn not observed; protoconch indicates planktotrophic development.

**Radula (Figures 9A, B):** Relative radular length 2.5–5.9. Rachidian: length/width 1.21–1.47; major cusp elongate, blunt or rounded at tip; 2 extra denticles at concave anterior edge. Lateral and inner marginal: major cusps elongate rectangular, blunt at tip. Outer marginal: 8–10 cusps.

**Habitat:** Rock faces, shallow rock pools, and among barnacles and mussels; in upper eulittoral; recorded on granite and volcanic conglomerate; usually on wave-exposed open coasts, apparently rare at sheltered sites. A study of zonation and temperature relations by Markel (1971) included both this species and *N. conspersa* (as *Littorina modesta*).



**Range (Figure 10):** Southern Baja California, southern Gulf of California, Mexico, Costa Rica, Islas Revillagigedo and Clipperton Atoll. Range limits: Bahía Magdalena, Baja California Sur (USNM 264568, 2 specimens); 4 km S of Todos Santos, Baja California Sur (USNM 794301); Bahía Santa Maria, near Cabo San Lucas, Baja California Sur (BMNH 20001187); Punta Pescadero, Baja California Sur (BMNH 20001188); Playa Coromuel, 3 km N of La Paz, Baja California Sur (BMNH 20001189, 1 specimen); Isla Espiritu Santo, Baja California Sur (USNM 538110, 1 specimen); Isla Carmen, Baja California Sur (USNM 558508, 1 specimen); Guaymas, Sonora (BMNH 20001190, 1 specimen; USNM 701409, 4 specimens); Topolobampo, Sinaloa (BMNH 20001191, 3 specimens); Mazatlán, Sinaloa (BMNH 20001192); Puerto Angel, Oaxaca (BMNH 20001192); Playa de Manuel Antonio, Puerto Quepos, Costa Rica (BMNH 20001194, 40 specimens); Isla Socorro (USNM 60648; KKK); Clipperton Atoll (KKK, 2 specimens). The species is rare in the Gulf of California (a total of only 12 specimens have been seen from north of La Paz and Mazatlán). There is only a single locality record from Central America, although 40 specimens were collected. The species is common on Isla Socorro (and was also listed from Isla Clarion by Emerson, 1995), but only two specimens are known from Clipperton Atoll, where it is probably an occasional immigrant (it was not recorded in a list of the mollusks by Emerson, 1994). As discussed in the Taxonomic History above, *N. modesta* was for long thought to occur in the northern Pacific, following the erroneous locality of Sitka given by Philippi (1846a, 1847).

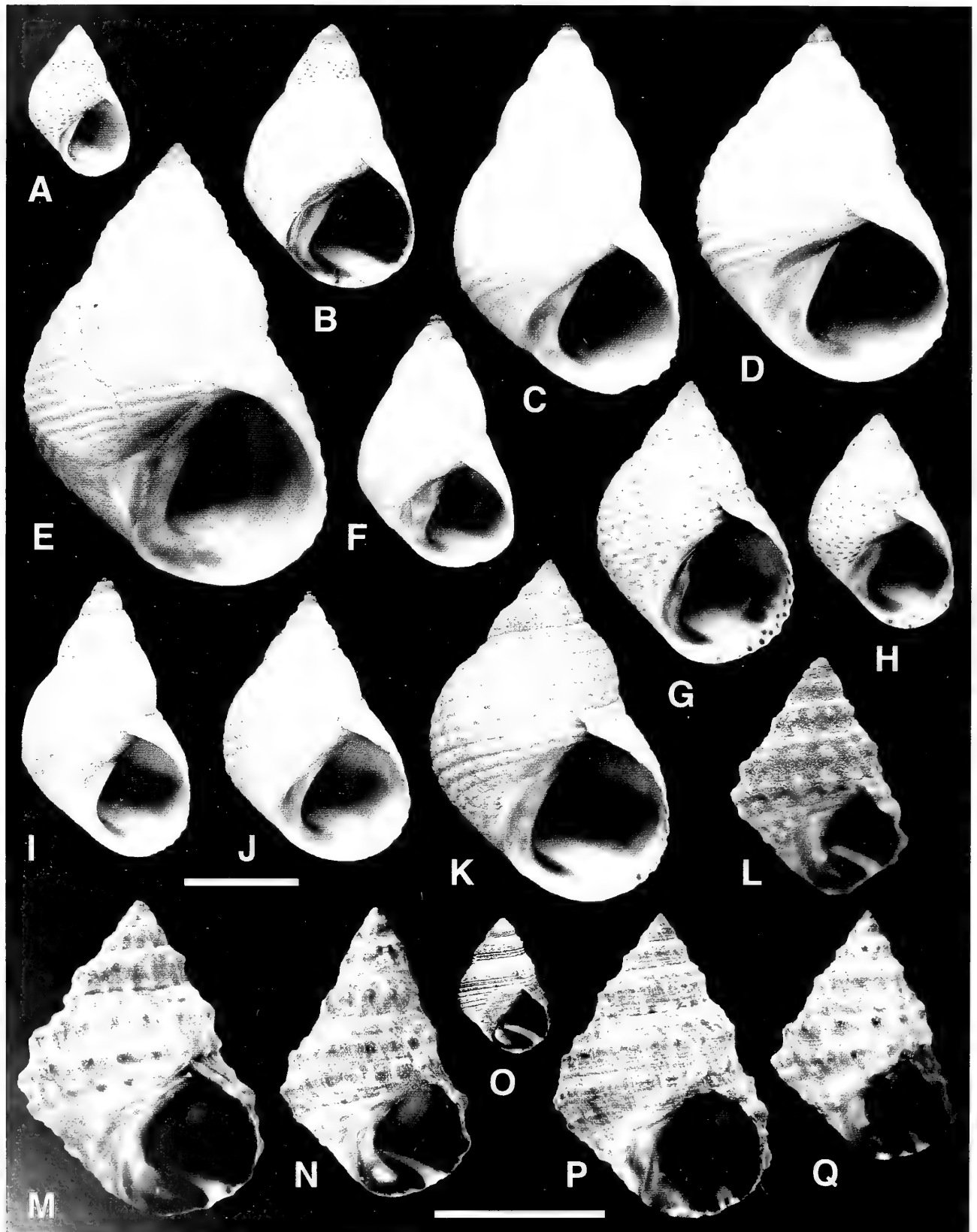
**Remarks:** This species is closely similar to the other member of the *modesta* group, *N. conspersa*; the characters most useful for discrimination are listed in Table 2. Geographical range is a useful criterion. So far, sympatric collections have been seen from only two localities, Puerto Angel (Oaxaca, Mexico) and near Puerto Quepos (Costa Rica), almost 1500 km apart; evidently both species sometimes disperse across the intervening Central American Gap (see Discussion). (If *Litorina albida* is correctly synonymized with *N. modesta*, El Realejo in Nicaragua is another site of sympatry.) In living or well preserved animals, the shape of the penis is diagnostic, but the differences are subtle and sometimes hard to discern if the filament is not clearly differentiated from the base (e.g., Figures 8C, E). Surprisingly, the coloration of the tentacles provides an equally accurate diagnostic character, with fine black transverse lines in *N. modesta* and a pair of longitudinal black lines in *N. conspersa*. The paraspermatzoa differ slightly, the rod-pieces of *N. modesta* being broader. No significant differences were observed in the pallial oviducts or radulae. Without the benefit of anatomical information, shell variation in this group is initially confusing, since the most obvious features of the variation, strength of sculpture and presence of colored

dots, do not separate the two taxa. Instead, a subtle and not entirely diagnostic character, the number of primary spiral grooves on the spire whorls, is most useful. There are several other minor differences: all-white shells occur only in *N. modesta*; if present, the dots are smaller, more numerous and grey or blackish brown (rather than orange-brown) in *N. modesta*; sculpture may be weak or strong in both, but grooves do not exceed the width of the ribs in *N. conspersa*; the spire whorls are slightly flatter in *N. conspersa*. Individually, these differences seem insignificant but, nevertheless, at the localities of sympatry, all specimens can be separated by shell characters alone, and the majority of unlocalized shells can be confidently assigned.

### *Nodilittorina conspersa* (Philippi, 1847)

(Figures 7G–K, 8G–M, O, R, S, 9C, 10)

- Littorina conspersa* Philippi, 1847:2:200–201, *Littorina* pl. 4, fig. 14 (Oceanus Pacificus Real Llejós in America centrali [El Realejo, Nicaragua]; neotype (here designated, 12.0 × 8.0 mm, El Realejo, Nicaragua) BMNH 199990405/1, seen, Figure 7J). Carpenter, 1857a:208. 230, 326 (in part, includes *N. modesta*). Carpenter, 1864b:538, 623 (in part, includes *N. modesta*). Wein-kauff, 1882:64–65, pl. 8, figs. 10, 11 (in part, includes *N. modesta*). Weinkauff, 1883:217 (in part, includes *N. modesta*). Stearns, 1891:327.
- Littorina conspersa*—C. B. Adams, 1852a:396. C. B. Adams, 1852b:172. Carpenter, 1857a:273. Mörch, 1860:69. Carpenter, 1863:352–353 (in part, includes *N. modesta*). Biolley, 1907:22. Morrison, 1946:10. Keen, 1958:282 (in part, includes *N. modesta*). Hertlein, 1963:239.
- Littorina (Melaraphe) conspersa*—H. & A. Adams, 1854:314. von Martens, 1900:577, 586 (in part, includes *N. modesta*).
- Littorina (Melaraphe) conspersa*—Carpenter, 1857b:346–347 (in part, includes *N. modesta*).
- Littorina (Melaraphe) aspera* var. *conspersa*—Tryon, 1887:249, pl. 44, figs. 82, 83 (in part, includes *N. modesta*).
- Littorina aspera conspersa*—Schwengel, 1938:2.
- Littorina puncticulata* Philippi, 1847:2:201, *Littorina* pl. 4, fig. 15 (Oceanus Pacificus Real Llejós in America centrali [El Realejo, Nicaragua]; types not found). Wein-kauff, 1882:63, pl. 8, fig. 9 (in part, includes *N. modesta*).
- Littorina puncticulata*—C. B. Adams, 1852a:396, 400. C. B. Adams, 1852b:176.
- Littorina (Melaraphe) puncticulata*—H. & A. Adams, 1854:314.
- Littorina (Melaraphe) conspersa* var. *puncticulata*—von Martens, 1900:577, 786–587 (in part, includes *N. modesta*).
- ? *Littorina* (? *Littorinopsis*) *conspersa* var. *fortisulcata* Nevill, 1885:138 (Nicaragua; *nomen nudum*).
- Littorina modesta*—Keen, 1971:366, fig. 183 (in part, includes *N. modesta*). Montoya, 1983:332 (in part, includes *N. modesta*). Finet, 1985:13 (not Philippi, 1846). Alamo & Valdivieso, 1987:26, fig. 39 (not Philippi, 1846). Alamo & Valdivieso, 1997:18, fig. 39 (not Philippi, 1846).



*Littorina modesta modesta*—Vermeij, 1973:324 (not Philippi, 1846).

*Littorina (Littorinopsis) modesta*—Abbott, 1974:69 (in part, includes *N. modesta*).

*Nodilittorina* (? *Fossarilittorina*) *modesta*—Reid, 1989a:98, pl. 2, fig. e (in part, includes *N. modesta*). Skoglund, 1992:16 (not Philippi, 1846). Kaiser, 1997:27 (not Philippi, 1846).

*Nodilittorina modesta*—Finet, 1994:18 (not Philippi, 1846).

**Taxonomic history:** No original type material of *Littorina conspersa* is known to exist. Nevertheless, there is no doubt as to its identity, since Philippi's (1847) figure clearly shows the relatively flat whorls and slightly patulous shape, and he accurately described the five grooves on the penultimate whorl and pair of divided ribs near the suture of the last whorl, thus differentiating it from the similar *N. modesta*. To stabilize the concept of this taxon, a neotype is designated. Philippi (1847) noted that the material from El Realejo, Nicaragua, on which his description was based, was obtained from Petit. The neotype is from the same type locality, and was collected by R. B. Hinds on the voyage of the *Sulphur* (1836–1842). It is possible that Philippi's material might have originated from this same source, since both Hinds and Petit were in contact with Cuming in London, but there is no direct evidence for this. The types of *Littorina puncticulata* are lost; it is included in the synonymy of *N. conspersa* (as first noted by Carpenter, 1857a) because of the four ribs on the penultimate whorl and the presence of a dotted pattern despite the strong sculpture (strongly sculptured *N. modesta* tend to be white); Philippi's (1847) description of a riblet in each groove on the last whorl could apply to either of the species in the *N. modesta* group.

The longstanding confusion of the names *conspersa* and *modesta* has been discussed in the Taxonomic History of *N. modesta*. Previously, very few authors have recognized more than one species in the *N. modesta* group in Central America. Philippi (1847; followed by Weinkauff, 1882) recognized three from the single locality El Realejo (Nicaragua) and believed a fourth, *Littorina modesta*, to be from Alaska and Mauritius. His *Littorina conspersa* was based on elongate, relatively weakly sculptured shells, while *Littorina puncticulata* was introduced for globular, ribbed examples now known to be a

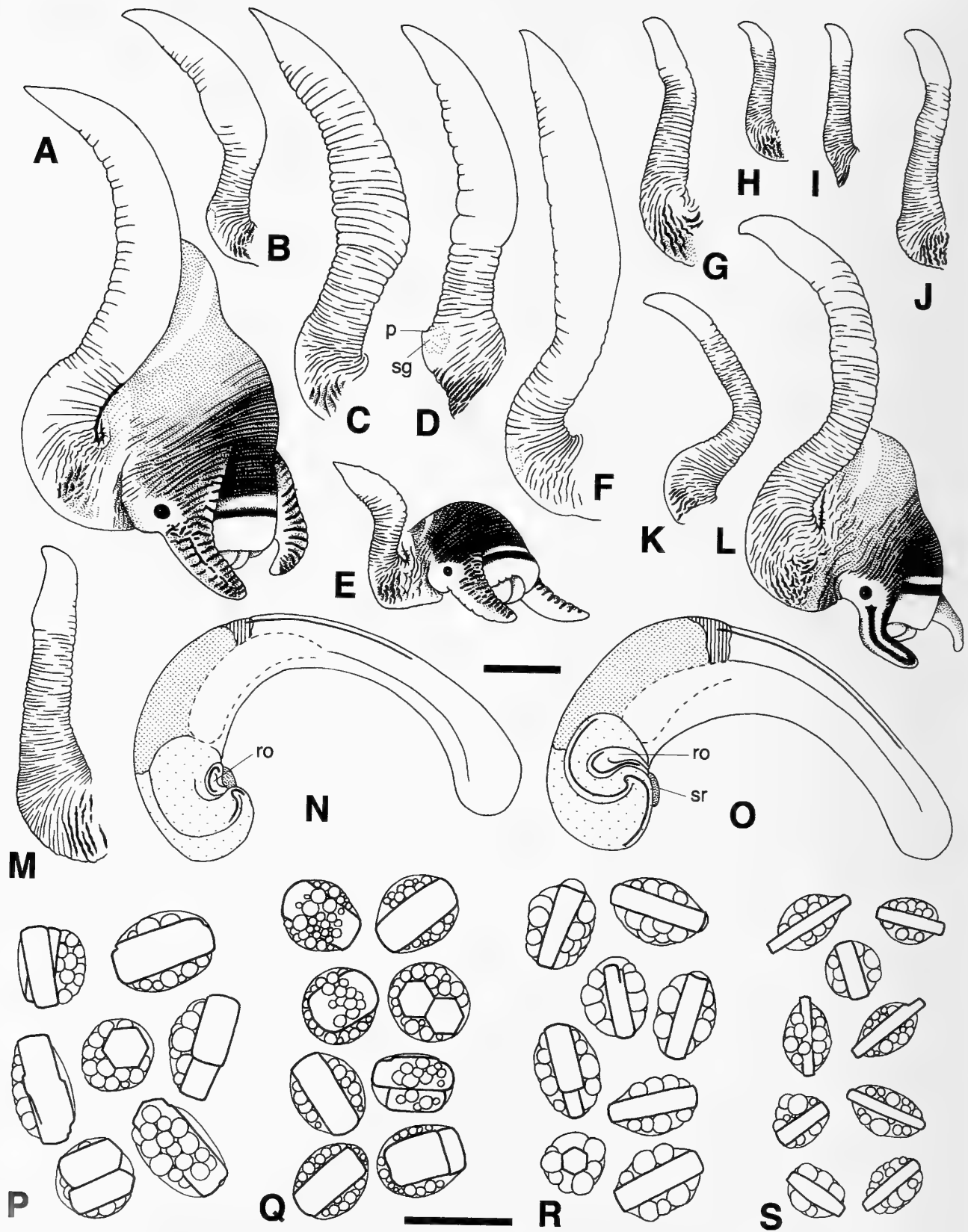
form of the same species (the third, *Littorina albida*, was probably an elongate, weakly sculptured form of *N. modesta*; see Taxonomic History of *N. modesta*). C. B. Adams (1852a, b) likewise separated more and less sculptured forms as *Littorina puncticulata* and *L. conspersa* (although he noted some intergradation); these were synonymized by Carpenter (1863). All subsequent authors recognized only a single species in the region (including Weinkauff, 1883), although von Martens (1900) still used *Littorina conspersa* var. *puncticulata* for strongly ribbed shells.

**Diagnosis:** Shell moderately large, spire whorls moderately flattened; 4–5 primary spiral grooves; sculpture of incised lines only, or with deep grooves 0.5–1 times rib width; pattern of small orange-brown dots. Tentacles with two longitudinal black lines. Penis with short filament, bluntly hooked at tip, glandular flange at base, no mamilliform gland.

**Material examined:** 53 lots (including 15 penes, 5 sperm samples, 10 pallial oviducts, 4 radulae).

**Shell (Figures 7G–K):** Mature shell height 4.4–18.2 mm. Shape high turbate to slightly patulous (H/B = 1.42–1.65, SH = 1.48–2.07); spire whorls moderately flattened, suture distinct; periphery of last whorl weakly angled. Columella straight, broad, slightly hollowed and pinched (sometimes with slight protruberance) at base; rarely a small imperforate, pseudo-umbilical area; eroded parietal area in larger shells. Sculpture of 4–5 (sometimes 6) primary spiral grooves on spire whorls; these may remain as incised lines only, numbering 8–10 above peripheral rib of last whorl (11–14 in total including basal grooves), but usually become slightly wider and deeper toward periphery; grooves rarely become obsolete on shoulder of last whorl; in strongly sculptured shells grooves are deeper throughout, separating rounded ribs on spire whorls, on last whorl ribs are raised (most strongly so near periphery), rounded, separated by grooves 0.5 to 1 times rib width, narrow interpolated rib may appear in 2 posterior grooves near suture (rarely in all grooves above periphery), or occasionally 1–3 posterior ribs become divided by a secondary groove, grooves on last whorl then up to 16 above peripheral rib (up to 23 in total including basal

Figure 7. Shells of *Nodilittorina modesta* (A–F), *N. conspersa* (G–K), and *N. galapagensis* (L–Q). A. Playa de Manuel Antonio, Puerto Quepos, Costa Rica (BMNH 20001194). B. Mazatlán, Sinaloa, Mexico (BMNH 20001192). C. 7 km NE of San José del Cabo, Baja California Sur, Mexico (BMNH 20001195). D. Locality unknown (BMNH 20001196). E. Lectotype of *Littorina modesta* Philippi, 1846; locality unknown (BMNH 1968224/1). F. Bahía Santa María, Baja California Sur, Mexico (BMNH 20001187). G. Same, Esmeraldas, Ecuador (BMNH 20001200). H. Tarcoles, Costa Rica (BMNH 20001205). I. Punta Chocollatera, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20001206). J. Neotype of *Littorina conspersa* Philippi, 1847; El Realejo, Nicaragua (BMNH 19990405/1). K. Bahía Chatham, Isla del Coco, Costa Rica (KLK). L. Holotype of *Littorina (Tectarius) galapagensis* Stearns, 1892; Isla Santiago, Galápagos Islands (USNM 102509). M. O. Q. Punta Estrada, Isla Santa Cruz, Galápagos Islands (M. BMNH 20001274; O. BMNH 20001275; Q. BMNH 20001276). N. P. Puerto Ayora, Isla Santa Cruz, Galápagos Islands (N. BMNH 20001273; P. BMNH 20001277). Scale bars A–K = 5 mm; L–Q = 5 mm.



grooves); spiral microstriae absent. Protoconch 0.31 mm diameter, sculptured by spiral ribs. Color white, with pale brown or lilac-grey apex; patterned with small orange-brown (sometimes grey-brown) spots, aligned in oblique (opisthocline) series (commonly less than 16 spots from peripheral rib to suture on last whorl, but up to 26); aperture orange-brown with 2 broad pale bands, external spotting showing through near margin; columella orange-brown to purple-brown, pillar sometimes white.

**Animal (Figure 8L):** Head black to pale grey, unpigmented stripe across black snout, tentacle with two longitudinal black lines, usually meeting close to tip, grey beneath; sides of foot black to pale grey. Opercular ratio 0.32–0.37. Penis (Figures 8G–M): narrow, vermiform; filament short (about 0.25–0.35 total length), with subepithelial glandular tissue, bluntly hooked tip, filament differentiated from base by slight constriction and lack of annular wrinkles (distinction sometimes unclear); sperm groove open to tip; base with fine annular wrinkles except at basal posterior edge with slight glandular flange (opaque subepithelial glandular tissue sometimes visible), base usually slightly pigmented. Euspermatozoa 54–71  $\mu$ m; paraspermatozoa (Figures 8R, S) round to oval; rod-pieces single (rarely two), filling cell or projecting, 9–22  $\mu$ m, blunt, hexagonal in section; granules large, spherical, distinct. Pallial oviduct (Figure 8O) with long straight section; large copulatory bursa opening near posterior end of straight section, extending back to albumen gland; small loop of renal oviduct projects into center of spiral of albumen gland. Spawn not observed; protoconch indicates planktotrophic development.

**Radula (Figure 9C):** Relative radular length 3.6–5.1. Rachidian: length/width 1.40–1.50; major cusp elongate, rounded at tip; 2 extra denticles usually present at concave anterior edge. Lateral and inner marginal: major cusps elongate rectangular, blunt or rounded at tip. Outer marginal: 8 cusps.

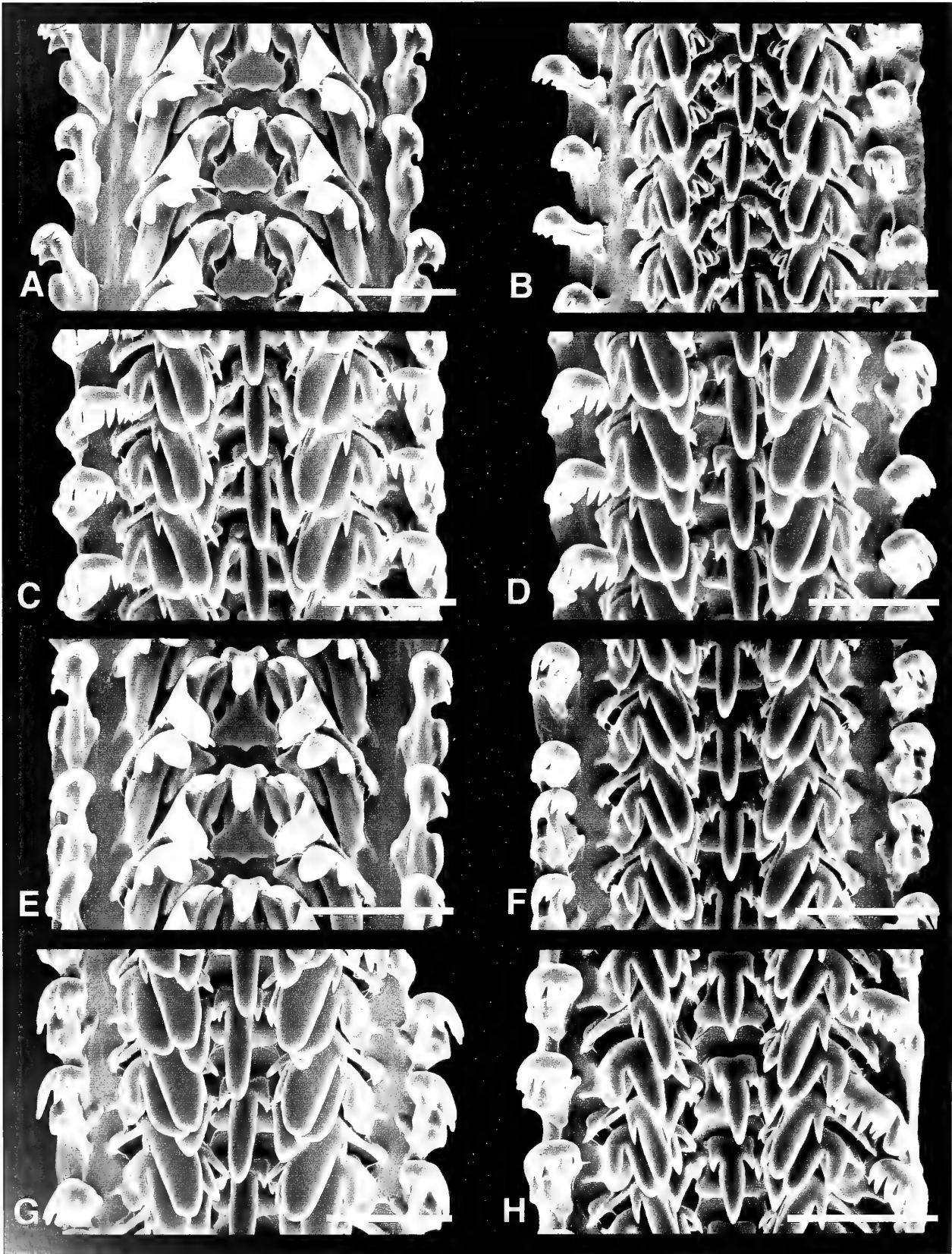
**Habitat:** Rock faces, and among barnacles and mussels;

in upper eulittoral and low littoral fringe, below level of sympatric *N. dubiosa* and *N. tenuistriata*; recorded on basalt, volcanic conglomerate, sandstone, mudstone, and concrete; usually on wave-exposed open coasts, scarce at sheltered and turbid sites. Bakus (1975) recorded it only in protected sites at Isla del Coco. For ecological studies and descriptions of zonation see Markel (1971, includes *N. modesta*), Cantera et al. (1979), Garrity & Levings (1981), Garrity (1984) (all as *Littorina modesta*).

**Range (Figure 10):** Oaxaca (Mexico), El Salvador to northern Peru, Isla del Coco and Galápagos Islands. Range limits: Puerto Angel, Oaxaca (BMNH 20001198, 7 specimens); Playa El Cucu, San Miguel, El Salvador (CAS, 4 specimens); Punta Amapala, El Salvador (USNM 780446); Isla del Coco, Costa Rica (BMNH 20001199; KLK); Isla de Malpelo, Colombia (USNM 122854; KLK); Punta San Francisco, Bahía Solano, Colombia (USNM 819734); Isla Gorgona and Isla Gorgonilla, Colombia (USNM 819735); Same, Esmeraldas, Ecuador (BMNH 20001200); Punta Carnero, Guayas, Ecuador (BMNH 20001201); 46 km from Caleta Mero, Tumbes, Peru (Alamo & Valdivieso, 1987, 1997); Paita, Piura, Peru (Stearns, 1891); Galápagos Islands (Isla Santiago, USNM 807235; Isla Santa Cruz, BMNH 20001202; Isla Española, BMNH 20001203; Isla San Cristobal, BMNH 20001204). At the most northerly site, Puerto Angel, this species is much less common than *N. modesta*. It is relatively frequent in the Galápagos Islands on suitably exposed shores and also at Isla del Coco. The accuracy of the most southerly record (Stearns, 1891) might be doubted, since the reliability of another in the same publication is questionable (see Range of *N. galapagensis*).

**Remarks:** See Remarks on *N. modesta* and Table 2 for discrimination from *N. conspersa*. Specimens of *N. conspersa* from the Galápagos Islands do not appear to differ anatomically from mainland examples, but their shells are slightly more tall-spired than most of the latter, and their

Figure 8. Penes (A–M), head pigmentation (A, E, L), pallial oviducts (N, O), and paraspermatozoa (P–S) of *Nodilittorina modesta* group: *N. modesta* (A–F, N, P, Q) and *N. conspersa* (G–M, O, R, S). A, Q. Mazatlán, Sinaloa, Mexico (BMNH 20001192; shell H = 10.0 mm). B. Playa de los Muertos, Puerto Vallarta, Jalisco, Mexico (BMNH 20001197; shell H = 7.8 mm). C, N, P. Bahía Santa María, Baja California Sur, Mexico (BMNH 20001187; shell H = 11.6 mm, 12.0 mm). D. Punta Pescadero, Baja California Sur, Mexico (BMNH 20001188; shell H = 11.1 mm). E. Playa de Manuel Antonio, Puerto Quepos, Costa Rica (BMNH 20001194; shell H = 6.2 mm). F. Puerto Angel, Oaxaca, Mexico (BMNH 20001193; shell H = 12.5 mm). G, J, S. Ballenita, Guayas, Ecuador (BMNH 20001207; shell H = 7.4 mm, 7.7 mm). H, I. Playa de Manuel Antonio, Puerto Quepos, Costa Rica (BMNH 20001208; shell H = 5.4 mm, 5.9 mm). K. Punta Estrada, Isla Santa Cruz, Galápagos Islands (BMNH 20001202; shell H = 7.2 mm). L. Puerto Angel, Oaxaca, Mexico (BMNH 20001198; shell H = 9.8 mm). M, R. Same, Esmeraldas, Ecuador (BMNH 20001200; shell H = 10.5 mm). O. Tarcoles, Costa Rica (BMNH 20001205; shell H = 11.2 mm). Abbreviations: p, papilla on penial base; ro, loop of renal oviduct projecting into albumen gland; sg, subepithelial glands in penial base (dotted line; sometimes visible by transparency); sr, seminal receptacle. Shading conventions as in Figures 3, 4. Scale bars A–O = 1 mm; P–S = 20  $\mu$ m.





whorls a little more rounded, which might indicate a degree of genetic differentiation of the island populations.

### The *Nodilittorina aspera* Group

Six species are included in this group: *N. aspera*, *N. tenuistriata*, *N. dubiosa*, *N. apicina*, *N. penicillata*, and *N. paytensis*. Superficially, the shells are very similar, and as a result these are perhaps the most confusing and difficult to identify of the *Nodilittorina* species of the region. These are medium to large members of the genus, with white to blue-grey shells, usually with a conspicuous dark pattern of axial stripes and, typically, a spiral black line, band or grey zone above the periphery which is prominent on the spire whorls, but fades on the last whorl. The aperture is brown, with two pale bands. The sculpture is of spiral grooves, which sometimes become wide and separated by prominent ribs.

Not surprisingly, considerable uncertainty has surrounded the taxonomy of these species. All but one (*N. tenuistriata* Reid, sp. nov.) were named before 1864, but this was a fortuitous result of limited availability of material and lack of appreciation of the range of shell variation. The first to be named was *Littorina aspera* Philippi, 1846, followed by *Littorina paytensis* Philippi, 1847. In 1851 Menke provisionally named *Littorina apicina*, but was unwilling to separate it as a distinct species from *L. aspera* in the same collection from Mazatlán. With a larger volume of material from Mazatlán at his disposal, Carpenter (1857b) correctly distinguished two species in this complex, *L. aspera* and *L. philippii* (= *N. apicina*) and later (1864a) described *Littorina penicillata* as a variety of the latter. Meanwhile, C. B. Adams (1852a, b) used the name *Littorina aspera* for large ribbed shells from Panama, whereas smaller smooth shells (now known to be conspecific) were doubtfully identified as *L. parvula* (a *nomen dubium* introduced by Philippi, 1849) and provisionally renamed *L. dubiosa*. Thus several early authors recognized two species in the complex, a ribbed form named *aspera* and a smaller, smoother shell variously named *apicina*, *dubiosa*, *parvula*, or *philippii*. In Mexico (Menke, 1851; Carpenter, 1857b, 1864a) this distinction did indeed correspond to that now made on anatomical grounds between *N. aspera* and *N. apicina*. However, the intraspecific variation in size and sculpture was not appreciated

and therefore farther south in Central America (where *N. apicina* is rare) the names *aspera* and *dubiosalparvulaphilippii* were applied to rough and smooth extremes of the single common species (for which the valid name is *N. dubiosa*) (C. B. Adams, 1852a, b; Carpenter, 1863). This distinction of two species contrasting in sculpture was continued in systematic revisions by Weinkauff (1882), von Martens (1900), and Keen (1958), in which *aspera* was applied to ribbed shells of both *N. aspera* and *N. dubiosa*, whereas *philippii/dubiosa* included a range of smoother forms (*N. apicina*, *N. penicillata*, *N. dubiosa*, and even *N. modesta* and *N. interrupta*). There was, however, an alternative tendency to combine these variable and troublesome shells as a single species, for which the earliest name *aspera* was employed (Weinkauff, 1883; Tryon, 1887; Keen, 1971; Abbott, 1974). Tryon (1887) even included the *N. modesta* group under the specific name *aspera*. In the literature of the past 30 years there has been no attempt to revise the *N. aspera* group, despite the observation by Keen (1971) that careful study might reveal a complex of species. The faunistic lists of areas of Mexico, Central America, and the Galápagos that appeared during the twentieth century have almost all listed only *aspera* (Biolley, 1907; Morrison, 1946; Hertlein, 1963; Montoya, 1983; Finet, 1985, 1994; Holguín & González, 1989; Emerson, 1995; Kaiser, 1997), although an exception was that of Pilsbry & Lowe (1932) in which the names *aspera*, *penicillata*, and *philippii* were listed. Curiously, the predominantly southern species *N. paytensis* has sometimes been recognized as distinct in revisions and worldwide lists (Weinkauff, 1882, 1883; Rosewater, 1970; Keen, 1971), and in the Peruvian literature (Vegas, 1968; Peña, 1971b; Alamo & Valdivieso, 1987, 1997; Paredes et al., 1999), despite its close resemblance to other members of the *N. aspera* group. If synonymized at all, it was combined with another (but not closely related) southern species, *N. araucana* (Tryon, 1887; Dall, 1909; Hertlein & Strong, 1955b; Reid, 1989a; Finet, 1994), or doubtfully reduced to a subspecies of *N. modesta* (Keen, 1971; Vermeij, 1973; Finet, 1985). The other southern species in the *N. aspera* group, *N. tenuistriata* Reid, sp. nov., has hitherto appeared only in Peruvian literature, identified as *Littorina aspera* (Alamo & Valdivieso, 1987, 1997; Paredes et al.,

←

Figure 9. Radulae of *Nodilittorina modesta* (A, B), *N. conspersa* (C), *N. dubiosa* (D), *N. aspera* (E, F), *N. tenuistriata* Reid, sp. nov. (G), and *N. penicillata* (H). A, B. Bahía, Santa María, Cabo San Lucas, Baja California Sur, Mexico (BMNH 20001187; two views of radula, flat and at 45°; shell H = 10.9 mm). C. Ballenita, Guayas, Ecuador (BMNH 20001207; at 45°; shell H = 7.9 mm). D. Punta Morales, Golfo de Nicoya, Costa Rica (BMNH 20001229; at 45°; shell H = 11.3 mm). E, F. Puerto Marques, Acapulco, Guerrero, Mexico (BMNH 20001217; two views of radula, flat and at 45°; shell H = 11.3 mm). G. Playa de Manuel Antonio, Puerto Quepos, Costa Rica (BMNH 20001223; at 45°; shell H = 11.2 mm). H. San Felipe, Baja California Norte, Mexico (BMNH 20001250; at 45°; shell H = 8.2 mm). Scale bars = 50 µm.



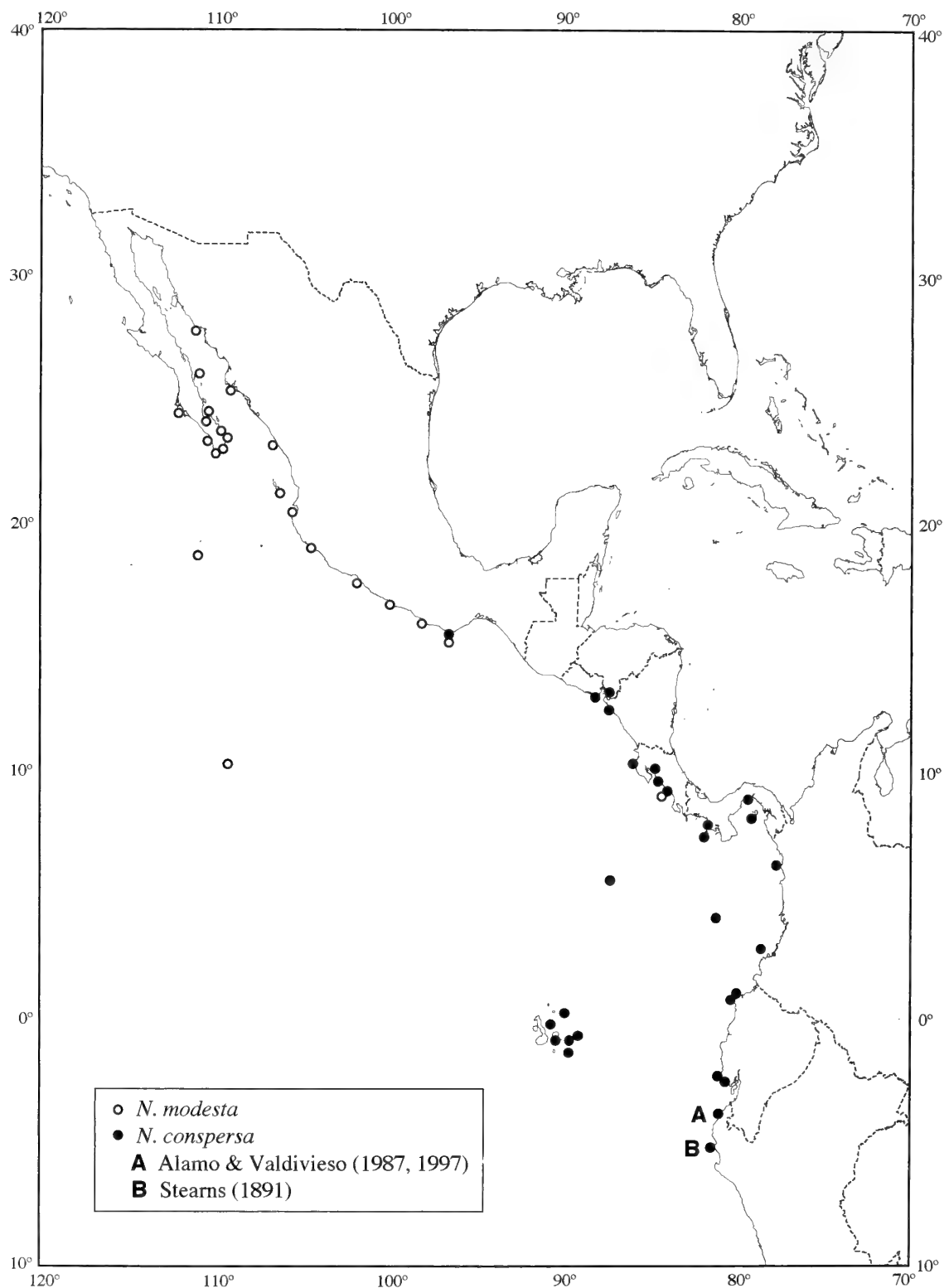


Figure 10. Geographical distribution of *Nodilittorina modesta* group (records based on material examined and quoted literature sources).

1999). In the two most recent worldwide lists of species of Littorinidae, Rosewater (1970) included *Littorina aspera*, *L. penicillata*, and *L. paytensis*, whereas Reid (1989a) gave only *N. aspera*, with *N. penicillata* and *N. paytensis* both of doubtful status.

As in the other species complexes of *Nodilittorina*, the crucial observations leading to discrimination of these difficult taxa have been the discovery of sympatric and syntopic occurrences, localities at which the distinctive shell types co-occur on rocks on the shore (though often at slightly different levels within the uppermost eulittoral and littoral fringe). At the southern tip of Baja California three species co-occur (*N. aspera*, *N. apicina*, *N. penicillata*); in southern Mexico two (*N. aspera*, *N. apicina*); in Nicaragua four (*N. aspera*, *N. tenuistriata*, *N. dubiosa*, *N. apicina*); in Costa Rica four (*N. tenuistriata*, *N. dubiosa*, *N. apicina*, *N. paytensis*); and in Ecuador three (*N. tenuistriata*, *N. apicina*, *N. paytensis*). Correlated with these shell types are differences in the shape of the penis, but these are more subtle than is often the case in littorinids, and are not always diagnostic. Other anatomical features, such as the paraspermatozoa and copulatory bursa, also provide discriminating characters in some cases, but radulae do not. The most useful characters for identification of species in this complex are summarized in Table 3.

Another key to the understanding of the *N. aspera* complex is the recognition that the strength of shell sculpture is variable in most species; that is, the width and depth of the spiral grooves varies, although their number is more constant. Early authors identified those large shells with strong ribs and wide grooves as *aspera*, and separated smaller smoother forms as *apicina*, *philippii*, *parvula*, and *dubiosa*, as discussed above. In fact, most species (except *N. tenuistriata*) can show almost completely smooth shells in dwarf or stunted forms, and strong spiral ribs are seen only on the last whorl of large examples of *N. aspera* and *N. dubiosa*. Surprisingly, in this group, shell color pattern is a more reliable guide for rapid identification in the field (Table 3), although in other members of the genus (e.g., *N. porcata* group) this is variable. The intensity of the dark pattern does nevertheless show variation and is especially dark in dwarf shells, which can be found for example in saline pools high in the eulittoral zone. As in the *N. porcata* group, it is suggested that this may be a case of ecophenotypic variation (see Discussion).

There is a parallel between the *N. aspera* group and the complex of black and white shells (informally known as the *N. ziczac* group) found in the Caribbean and western Atlantic, which consists of at least four (Reid, 1989a) or as many as six (Bandel & Kadolsky, 1982) species. Whether they share common ancestry is not yet known. When the origin of specimens is not known, confusion can arise; for example, von Martens (1900) erroneously described a Caribbean species (*N. interrupta*) as *Littorina*

*philippii* var. *latistrigata*, believing it to have originated from the Pacific coast of Costa Rica.

### *Nodilittorina aspera* (Philippi, 1846)

(Figures 9E, F, 11A–H, 13A–F, 15A, J, K, 17)

*Littorina aspera* Philippi, 1846a:139 (Sitka, New Albion [Alaska]; Mexico; Conchagua, San Salvador [El Salvador]; here restricted to Mazatlán, Mexico; lectotype (here designated, 16.6 × 10.9 mm) BMNH 1968217/1, seen, Figure 11A; 3 paralectotypes BMNH 1968217/2, seen). Middendorff, 1849:394. Carpenter, 1857a:162, 216, 224, 286. ? Troschel, 1858:135, pl. 11, fig. 4 (radula). Keen, 1958:282, ? fig. 173 right (in part, includes *N. dubiosa*). Keen, 1971:365, fig. 181 (part) (in part, includes *N. apicina*, *N. penicillata*, *N. dubiosa*, *N. modesta*, *N. interrupta*). Holguín & González, 1989:114, fig.

*Littorina aspera*—Philippi, 1847:2:200 (in part, fig. is *N. dubiosa*). Menke, 1851:163–164 (in part, includes *N. apicina*). Carpenter, 1857a:235, 237, 257, 326, 348 (in part, includes *N. dubiosa*). Carpenter, 1864b:623 (in part, includes *N. dubiosa*). Weinkauff, 1882:60–61 (in part, figs. are *N. dubiosa*). Weinkauff, 1883:220 (in part, includes *N. apicina*, *N. dubiosa*, *N. penicillata*). Carpenter, in Brann, 1966:pl. 38, fig. 397.

*Littorina (Melaraphe) aspera*—H. & A. Adams, 1854:314. von Martens, 1900:577, 587, ? pl. 43, fig. 15 (in part, includes *N. dubiosa*, *N. apicina*). Tryon, 1887:249, pl. 45, fig. 87 (in part, includes *N. modesta*, *N. conspersa*, *N. apicina*, *N. penicillata*).

*Littorina (Melaraphe) aspera*—Carpenter, 1857b:348–349 (includes *N. dubiosa*).

*Littorina (Austrolittorina) aspera*—Rosewater, 1970:423. Abbott, 1974:69, fig. 561, pl. 3, fig. 561 (in part, includes *N. dubiosa*, *N. apicina*, *N. penicillata*).

*Nodilittorina (Nodilittorina) aspera*—Reid, 1989a:99 (in part, includes *N. dubiosa*, possibly *N. penicillata*). Skoglund, 1992:15 (in part, includes *N. dubiosa*, possibly *N. penicillata*).

*Nodilittorina aspera*—Emerson, 1995:13.

*Littorina irrorata*—Reeve, 1857:sp. 56, pl. 11, fig. 56a, b (not *Turbo irroratus* Say, 1822 = *Littoraria irrorata*).

**Taxonomic history:** Of the three collections mentioned by Philippi (1846a) in the original description of *aspera*, only that from Sitka has been located, but the locality is obviously erroneous (Carpenter, 1857a). Philippi apparently included the closely similar southern species *N. dubiosa* in his concept of the taxon, since his figure (Philippi, 1847) of *N. aspera* appears to represent *N. dubiosa*. To fix the concept of this taxon, a lectotype is designated from the extant syntypes, and the type locality is here restricted to Mazatlán.

This species was the first of the *N. aspera* group to be described and the name has remained one of the most familiar in the eastern Pacific fauna. Examples of this species have never been figured or described under any other name, with the exception of Reeve's (1857) "*Littorina irrorata*" (an error first noted by von Martens, 1900). Nevertheless, most authors have had too broad a

Table 3  
Summary of the most useful characters for the identification of the six species of the *Nodilittorina aspera* group.

Character	<i>N. aspera</i>	<i>N. tenuistriata</i>	<i>N. dubiosa</i>	<i>N. apicina</i>	<i>N. penicillata</i>	<i>N. paytensis</i>
1. Geographical range	S Baja California to S Mexico, Nicaragua	Nicaragua to N Peru	El Salvador to Colombia, Isla del Coco, Galápagos Is	S Baja California to N Ecuador	S Baja California, Gulf of California	Costa Rica to N Peru
2. Shell						
—spire profile	concave	straight	straight or concave	convex	straight	straight
—columella	concave	concave	concave	long, straight	concave	straight
—grooves above periphery of last whorl	7–10 deep wide grooves (up to 3 times rib width)	10–15 grooves (up to same width as ribs at periphery)	7–11 deep wide grooves (up to twice rib width) or incised lines mainly in stunted forms	9–12 incised lines or narrow grooves (rarely to half rib width)	8–11 incised lines	11–23 incised lines
—smooth form	in stunted forms	absent	broad blue-grey zone above periphery, line below periphery	rare	rare	frequent
—dark spiral bands (usually darkest on spire whorls)	broad black band above periphery on spire, black line below periphery	broad black band above periphery, black band below periphery	narrow oblique stripes	indistinct grey zone above periphery	1 narrow line above and 1 below periphery	broad brown band above periphery
—axial pattern	slightly oblique stripes	oblique axial lines	broad, smooth, tapering large small to medium	oblique or zigzag lines or stripes, or tessellation	oblique or waved lines	smudged brown dots
3. Penis						
—filament	smooth, tapering	wrinkled, tapering	broad, smooth, tapering large small to medium	long, wrinkled, tapering medium medium, forming a lobe	broad, smooth, pointed medium medium, forming a lobe	wrinkled, tapering, pointed medium to large small to medium
—mamilliform gland	medium	medium large				
—glandular disc	medium					

concept of this taxon, including with it various others from the *N. aspera* complex. In the early literature, most authors discriminated two species in the complex, one larger and more strongly sculptured (to which the name *aspera* was applied), the other smaller and relatively smooth (see Taxonomic History of *N. apicina* and *N. penicillata*). For the first time, it is shown here that the larger, more sculptured shells comprise three species, *N. aspera* s.s., *N. dubiosa*, and *N. tenuistriata*. Of these, the last is relatively uncommon in Central America and has been specifically referred to (as *aspera*) only in the Peruvian literature (Alamo & Valdivieso, 1987, 1997; Paredes et al., 1999). The other two have, however, been widely confused, although their largely allopatric distribution assists when synonyms are compiled (see also Taxonomic History of *N. dubiosa*). The use of the name *aspera* for all species in the *N. aspera* group, regardless of sculpture and size, dates from Weinkauff (1883) and Tryon (1887, who also included the *N. modesta* group). This practice was followed by Keen (1971), Abbott (1974), and Reid (1989a).

**Diagnosis:** Shell large, spire whorls slightly rounded, spire profile often slightly concave; 5–7 primary spiral grooves; 7–10 grooves above periphery of last whorl; sculpture of deep grooves up to 3 times rib width on last whorl of large shells, but only incised lines on small shells; white with brown axial stripes and (most obvious on spire whorls) broad spiral black band just above periphery. Penis with gradually tapering filament; mamilliform gland and glandular disc of similar size, on prominent projection of base.

**Material examined:** 47 lots (including 23 penes, 2 sperm samples, 4 pallial oviducts, 4 radulae).

**Shell (Figures 11A–H):** Mature shell height 5.0–22.0 mm. Shape high turbate ( $H/B = 1.27$ – $1.83$ ,  $SH = 1.43$ – $2.00$ ); spire whorls slightly rounded, suture distinct; spire profile usually slightly concave, giving slight onion shape; periphery of last whorl weakly angled. Columella concave, hollowed and slightly pinched at base; small eroded parietal area. Sculpture of (4) 5–7 primary spiral grooves on spire whorls; ribs subequal, or slightly wider at suture and periphery; 7–10 grooves above periphery of last whorl, secondary sculpture usually absent, but rarely 1–2 narrow secondary ribs develop posteriorly; on last whorl grooves enlarge to 1–3 times width of intervening ribs, which become narrow, sharply raised cords; in dwarf forms, the typical wide grooves do not develop, grooves remain as impressed lines, or become faint; spiral microstriae absent. Protoconch 0.31 mm diameter, 2.5 whorls. Color white, with slightly oblique or wavy axial brown stripes; on lower half of spire whorls a broad grey to black spiral band; on last whorl axial pattern may become less distinct except at suture, and broad grey band above periphery may also become faint; a narrow black

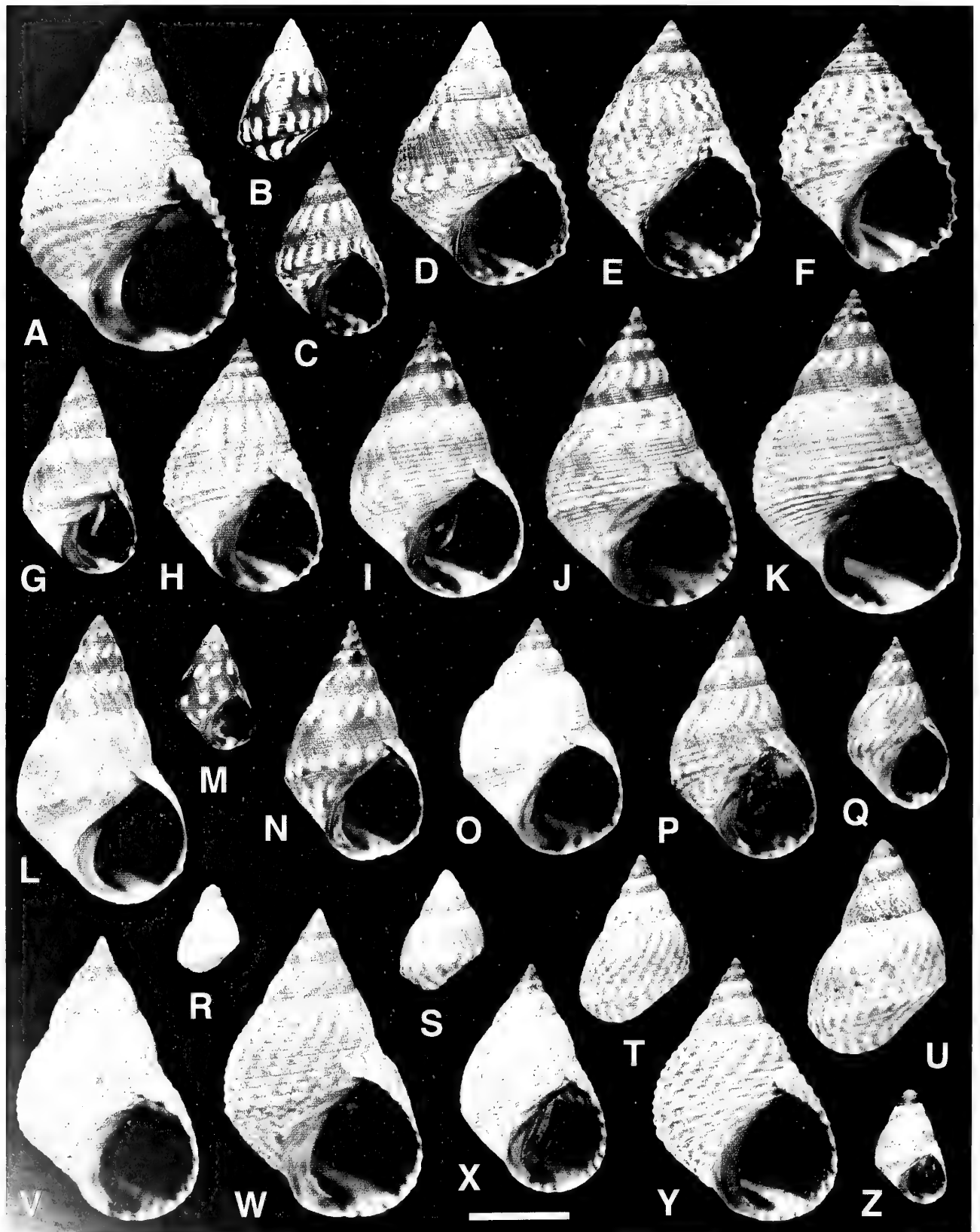
band is also present on base just below periphery; dwarf shells (Figure 11B) usually show striking pattern of black axial stripes and band above and below periphery; aperture brown, with 2 pale spiral bands at base and shoulder; columella brown.

**Animal:** Head grey to black, no unpigmented stripe across snout, tentacle pale at base and around eye, with two longitudinal black stripes, and black spot at tip; sides of foot pale grey to black. Opercular ratio 0.33–0.40. Penis (Figures 13A–F): filament moderately long, gradually tapering, smooth, thickened and glandular at base, 0.6–0.8 total length; sperm groove open to tip; mamilliform gland and glandular disc of similar size, on well developed projection of base; penis unpigmented or only slightly pigmented at base. Euspermatozoa not seen; paraspermatozoa (Figures 15J, K) 18–20  $\mu\text{m}$ , oval, with large round granules, single stout rod-pieces (sometimes composed of smaller rod-shaped elements) fill cells or project slightly. Pallial oviduct (Figure 15A) with large copulatory bursa opening at half length of straight section and extending back to albumen gland. Spawn not observed; protoconch indicates planktotrophic development.

**Radula (Figures 9E, F):** Relative radular length 10.2–15.7. Rachidian: length/width 1.50–1.79; major cusp elongate, rounded at tip. Lateral and inner marginal: major cusps elongate, rounded at tip. Outer marginal: 7–9 cusps.

**Habitat:** Clustered in crevices and on bare rock in littoral fringe; above water level at margins of shallow pools at top of eulittoral; largely restricted to exposed and moderately exposed coasts in oceanic situations; recorded on granite and concrete; usually abundant. Overlapping with *N. apicina*, but extending farther into littoral fringe.

**Range (Figure 17):** Southwestern Baja California to Oaxaca (Mexico), Islas Revillagigedo, Nicaragua. Range limits: Laguna San Ignacio, Baja California Sur (USNM 130599, 1 specimen); Bahía Magdalena, Baja California Sur (USNM 264566, 819879, 1 specimen each); Punta Lobos, Todos Santos, Baja California Sur (personal observation); 7 km N of San José del Cabo, Baja California Sur (BMNH 2001209); Punta Doble, Sonora (KLK, 1 specimen); Topolobampo, Sinaloa (BMNH 20001210, 1 specimen); Mazatlán, Sinaloa (BMNH 20001211); Bahía Ventosa, Golfo de Tehuantepec, Oaxaca (USNM 60449, 4 specimens); Salina Cruz, Golfo de Tehuantepec, Oaxaca (LACM 67-97-30, 3 specimens); Bahía Henslow, Isla Socorro, Islas Revillagigedo (KLK, 1 specimen); Corinto, Nicaragua (LACM 149775, 149776, 1 and 6 specimens); Nicaragua (CAS 122365, 2 specimens). This species is apparently very rare in the Gulf of California, from which only two specimens are known. It is, however, common on the southwestern coast of Baja California and from Mazatlán southward. Material from Nicaragua is scarce in collections; the two lots in LACM are apparently re-



liable and further supported by von Martens' (1900) figure of a specimen from El Salvador. Only a single specimen has been seen from Isla Socorro, although the name appears in lists from the island (Mille-Pagaza et al., 1994; Emerson, 1995).

**Remarks:** Since the original description of this species (Philippi, 1846a; see Taxonomic History above) the name has been applied to all large eastern Pacific littorinids with sharp ribs separated by wide grooves, and therefore also included large shells of *N. dubiosa* (distributed from El Salvador to Colombia). This similarity is, however, superficial and there are small but consistent differences in shell coloration, penial anatomy and habitat (see Remarks on *N. dubiosa*). The new species *N. tenuistriata* is separated from *N. aspera* by its finer sculpture (7–10 primary spiral grooves on spire whorls, 10–15 narrow grooves above periphery of last whorl), but similarities in shell coloration, paraspermatozoa, and habitat suggest a close relationship (see Remarks of *N. tenuistriata*). The known geographical distribution of *N. tenuistriata* just meets that of *N. aspera* at Corinto (Nicaragua) and extends to Peru; their distinguishing shell characters remain distinct at the one known locality of sympatry.

The distinctive coloration of *N. aspera* remains rather constant throughout its range. However, adult size varies widely and only shells of moderate and large size develop the strong sculpture of narrow ribs by which this species (together with *N. dubiosa*) has previously been characterized. Dwarf specimens (Figure 11B) are found in shallow pools at the top of the eulittoral zone, and in these small shells the sculpture is weak or almost absent. Dwarf specimens show a striking black pattern on a white ground. Similar variation is seen in dwarf examples of *N. apicina* from the same microhabitat; in both species occasional shells show an abrupt change to a more normal sculpture and pattern, implying an ecophenotypic component to the variation.

The radula is extraordinarily long, sometimes exceeding 15 times the length of the shell.

*Nodilittorina tenuistriata* Reid, sp. nov.

(Figures 9G, 11I–O, 13G–L, 15B, L, M, 17)

*Littorina* (*Austrolittorina*) *aspera*—Alamo & Valdivieso, 1987:25, fig. 35. Alamo & Valdivieso, 1997:17, fig. 35. Paredes et al., 1999:22. (All not Philippi, 1846).

**Etymology:** Latin: “finely striated,” describing the characteristic sculpture.

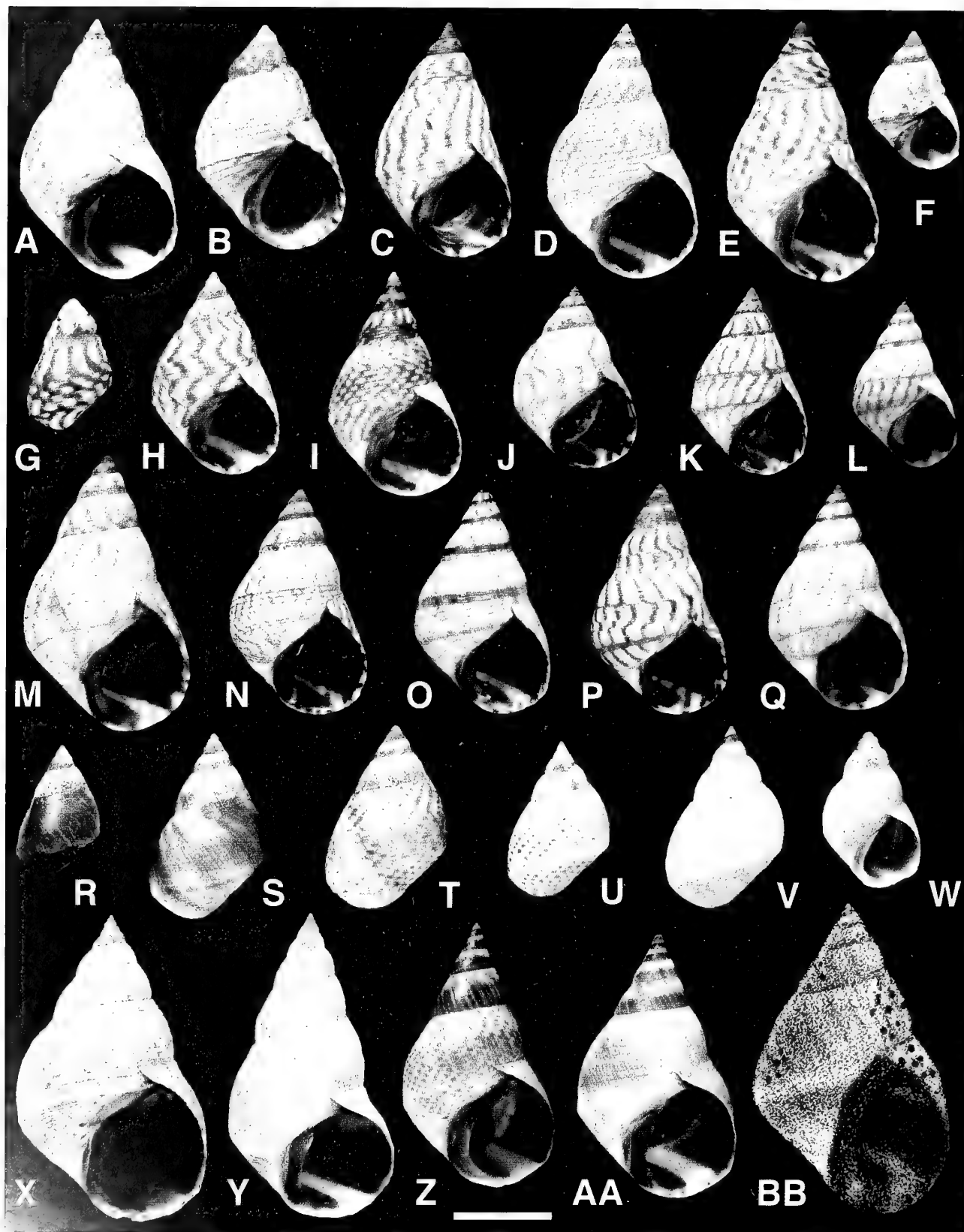
**Types:** Holotype BMNH 20000312 (Figure 11I). 28 paratypes BMNH 20000310 (Figure 11L, M). 100 paratypes BMNH 20000314 (ethanol). 4 paratypes USNM 894293. Type locality: Punta Chocolatera, Peninsula Santa Elena, Guayas Province, Ecuador.

**Taxonomic history:** This species has been almost entirely ignored in the literature. It has a wide distribution from Nicaragua to Peru. However, in Central America it is relatively uncommon, and no authors working in the area have distinguished it from the abundant *N. dubiosa*, whereas the littorinids of Colombia and Ecuador have scarcely been studied. Only in Peru (where *N. dubiosa* does not occur) has this species been specifically referred to, and then identified as *aspera* (Alamo & Valdivieso, 1987, 1997; Paredes et al., 1999).

Von Martens (1900) introduced the name *Littorina philippii* var. *latistrigata* for shells superficially similar to this species, white with oblique black axial stripes and spiral black band, although the sculpture was described as faint. The type locality was given as Punta Arenas, western Costa Rica. Examination of two syntypes in MNB has confirmed that they belong to the Caribbean species *N. interrupta*, characterized by waved or zigzag axial stripes, fine or obsolete sculpture, and raised dark brown inner lip of the aperture adjacent to the columella. Von Martens' type locality must be regarded as erroneous, but the species does occur on the eastern coast of Costa Rica.

**Diagnosis:** Shell large or small, whorls moderately rounded or slightly shouldered, spire profile usually

Figure 11. Shells of *Nodilittorina aspera* group: *N. aspera* (A–H), *N. tenuistriata* Reid, sp. nov. (I–O), and *N. dubiosa* (P–Z). A. Lectotype of *Littorina aspera* Philippi, 1846; locality unknown (BMNH 1968217/1). B, G. Bahía Santa María, Baja California Sur, Mexico (BMNH 20001212). C, E, H. Puerto Angel, Oaxaca, Mexico (C, H, BMNH 20001213; E, BMNH 20001214). D. Boca de Tomatlán, Jalisco, Mexico (BMNH 20001215). F. Playa de los Muertos, Puerto Vallarta, Jalisco, Mexico (BMNH 20001216). I. Holotype of *N. tenuistriata* Reid, sp. nov.; Punta Chocolatera, Peninsula Santa Elena, Guayas, Ecuador (BMNH 2000312). J, O. Tarcoles, Costa Rica (BMNH 20001224). K. Muisne, Esmeraldas, Ecuador (BMNH 20001225). L, M. Paratypes of *N. tenuistriata* Reid, sp. nov.; Punta Chocolatera, Peninsula Santa Elena, Guayas, Ecuador (BMNH 2000313). N. Same, Esmeraldas, Ecuador (BMNH 20001226). P. Isla San José, Islas Perlas, Panama (USNM 588051). Q. Punta Pitt, Isla San Cristobal, Galápagos Islands (BMNH 20001227). R. Coco, W of Liberia, Costa Rica (BMNH 20001228). S. San Juan del Sur, Nicaragua (USNM 23304). T, Y. Punta Morales, Golfo de Nicoya, Costa Rica (BMNH 20001229). U. Nicaragua (USNM 46973). V. Isla San José, Islas Perlas, Panama (USNM 587787). W. Bahía Chatham, Isla del Coco, Costa Rica (KLK). X. Tarcoles, Costa Rica (BMNH 20001230). Z. Lectotype of *Littorina dubiosa* C. B. Adams, 1852; Isla Taboga, Panama (MCZ 186573). Scale bar = 5 mm.





straight; 7–10 primary spiral grooves; 10–15 grooves above periphery of last whorl; sculpture of numerous fine grooves (10–15 above periphery of last whorl); white with oblique, grey-brown, axial lines and broad spiral grey to black band just above periphery (indistinct on last whorl). Penis with wrinkled filament roundly tapered at tip; mamilliform gland and large glandular disc borne on long projection of base.

**Material examined:** 31 lots (including 17 penes, 9 sperm samples, 4 pallial oviducts, 5 radulae).

**Shell (Figures 11I–O):** Mature shell height 5.0–19.6 mm. Shape high-turbinate to elongate ( $H/B = 1.39$ – $1.78$ ,  $SH = 1.47$ – $1.95$ ); spire whorls moderately rounded, suture distinct; spire profile usually straight, sometimes slightly concave; last whorl slightly shouldered, periphery angled. Columella concave, hollowed, and slightly pinched at base; eroded parietal area. Sculpture of 7–10 primary spiral grooves on spire whorls; primary ribs subequal, slightly wider toward suture; 10–15 (17) grooves above periphery of last whorl; peripheral rib raised and twice as wide as ribs above and below; secondary sculpture may be absent, or single narrow secondary riblets appear in grooves near suture and periphery; sculpture never becomes obsolete; on last whorl grooves close to periphery enlarge to 0.5–1 times width of intervening ribs, but others remain narrow; spiral microstriae absent. Protoconch not seen. Ground color white to pale blue-grey, with oblique brown, grey, or black stripes, interrupted by broad grey to black spiral band just above periphery and another on base; spiral bands become paler on last whorl, remaining as blue-grey zones; in dwarf forms (Figure 11M) a striking pattern of black stripes and bands on white ground; aperture brown, external pattern showing through, with 2 pale spiral bands at base and shoulder; columella brown.

**Animal:** Head grey to black, no unpigmented stripe across snout, tentacle pale around eye and sometimes at inside of base, with two longitudinal black stripes meeting at black tip; sides of foot grey or black speckled.

Opercular ratio 0.34–0.40. Penis (Figures 13G–L): filament moderately long, tapering toward rounded tip, with annular wrinkles on lower half, glandular, 0.6–0.8 total length; sperm groove open to tip; glandular disc large (usually larger than mamilliform gland), projecting as a lobe, borne with mamilliform gland on long projection of base (as long as filament, in well relaxed specimens); penis unpigmented or only slightly pigmented at base. Euspermatozoa 54–75  $\mu\text{m}$ ; paraspermatozoa (Figures 15L, M) oval with single (sometimes 2–3 if narrow) blunt or rounded rod, 10–34  $\mu\text{m}$ , projecting from cell, cytoplasm packed with large spherical granules. Pallial oviduct (Figure 15B) with large copulatory bursa opening at half length of straight section and extending back to albumen gland. Spawn not observed.

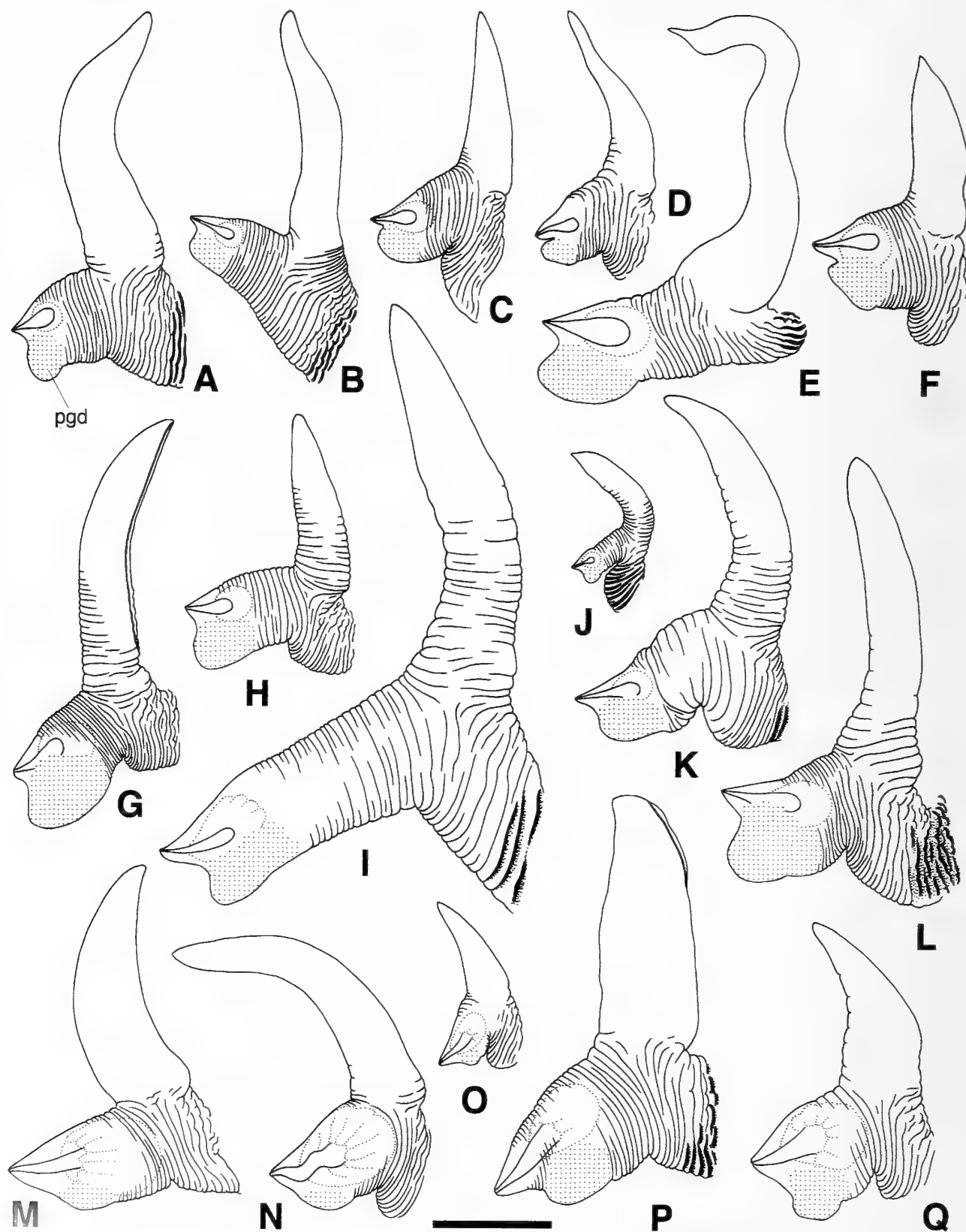
**Radula (Figure 9G):** Relative radular length 3.6–7.7. Rachidian: length/width 1.38–1.64; major cusp elongate, rounded at tip. Lateral and inner marginal: major cusps elongate, rounded at tip. Outer marginal: 6–7 cusps.

**Habitat:** Common on cliffs on exposed oceanic coasts; in littoral fringe, especially at sites of freshwater seepage; rare on rocks and piers on sheltered or muddy coasts; recorded on volcanic conglomerate, sandstone, mudstone, soil, concrete. Various microsympatric with *N. dubiosa*, *N. apicina*, and *N. paytensis* in different parts of its range, but extends higher up the shore than these.

**Range (Figure 17):** Nicaragua to northern Peru. Range limits: Corinto, Nicaragua (LACM 149776, 1 specimen); Playa del Coco, Costa Rica (G. J. Vermeij Collection); Punta Morales, Golfo de Nicoya, Costa Rica (BMNH 20001220); Fort Amador, Panama (USNM 732997, 1 specimen); Isla Gorgona, Colombia (USNM 819732, 3 specimens); Atacames, Esmeraldas, Ecuador (BMNH 20001221); Peninsula Santa Elena, Guayas, Ecuador (Punta Chokolatera, BMNH 20000314; Anconcito, BMNH 20001222); Paita, Piura, Peru (Alamo & Valdivieso, 1987, 1997; as *Littorina aspera*). This species is moderately common in Costa Rica, but much more so in Ecuador. Only a single specimen has been recorded from

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Figure 12. Shells of *Nodilittorina aspera* group (continued): *N. apicina* (A–I), *N. penicillata* (J–Q), and *N. paytensis* (R–BB). A. Same, Ecuador (BMNH 20001236). B. Lectotype of *Littorina philippii* Carpenter, 1857; Mazatlán, Sinaloa, Mexico (BMNH 1857.6.4.1682/1). C, E. Puerto Marques, Acapulco, Guerrero, Mexico (BMNH 20001237). D. Punta Morales, Golfo de Nicoya, Costa Rica (BMNH 20001238). E. Lectotype of *Littorina apicina* Menke, 1851; Mazatlán, Sinaloa, Mexico (SMF 314713/4). G. Boca de Tomatlán, Jalisco, Mexico (BMNH 20001239). H. La Manzanilla, 10 km N of Melaque, Colima, Mexico (BMNH 20001240). I. Atacames, Esmeraldas, Ecuador (BMNH 20001235). J, M, Q. Topolobampo, Sinaloa, Mexico (BMNH 20001247). K. Cabo San Lucas, Baja California Sur, Mexico (BMNH 20001244). L. Lectotype of *Littorina penicillata* Carpenter, 1864; Cabo San Lucas, Baja California Sur, Mexico (USNM 4058). N–P. San Felipe, Baja California Norte, Mexico (BMNH 20001245). R. Punta Carnero, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20001256). S–U. Ballenita, Guayas, Ecuador (BMNH 20001257). V. Playa de Manuel Antonio, Puerto Quepos, Costa Rica (BMNH 20001252). W. Mancora, Peru (USNM 663988). X. Muisne, Esmeraldas, Ecuador (BMNH 20001258). Y–AA. Punta Chokolatera, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20001259). BB. Lectotype figure of *Littorina paytensis* Philippi, 1847; Paita, Peru (Philippi, 1847: *Littorina* pl. 3, fig. 25). Scale bar = 5 mm.



Panama despite abundant littorinid collections from the area.

**Remarks:** This species shows a range of shell variation comparable with that seen in *N. aspera* and *N. apicina*; dwarf specimens are not only smaller but also show weaker sculpture, a striking black and white color pattern, and a slightly convex (domed) profile (Figure 11M). Some large specimens show early whorls of this dwarf form (Figure 11L; note change in spire profile and coloration), suggesting that ecophenotypic plasticity may be responsible. Habitat details for dwarf forms are lacking, but by analogy with these other species it is likely that they are found in unfavorable conditions, such as pools with fluctuating salinity high on the shore. Dwarf forms are presumably produced when growth is slow, whereas rapid growth results in shells that are paler, with concave spire profile and larger adult size.

For much of its range, *N. tenuistriata* is sympatric with *N. dubiosa*, and there is therefore no doubt that these two are distinct (for discrimination, see Remarks on *N. dubiosa*). Their typical habitats differ, *N. tenuistriata* being more frequent on exposed coasts, and *N. dubiosa* on relatively sheltered shores; nevertheless, they can be micro-sympatric on shores of moderate exposure, for example in Costa Rica. Interestingly, this species is very rare in the Gulf of Panama, possibly connected with eutrophic conditions there. This species exhibits an oceanic type of distribution. Curiously, on oceanic coasts it is most abundant at the very top of the shore where there is freshwater seepage from cliffs or hillsides.

Its relationship to *N. aspera*, recorded from Mexico to Nicaragua, is more problematic. The shell shape, size, and color pattern (with a broad peripheral dark band, especially on spire and in dwarf shells) are closely similar. Nevertheless, all shells (including dwarf forms) can be readily separated by their sculpture, which is of finer and more numerous grooves in *N. tenuistriata* (7–10 primary grooves on spire whorls, 10–15 narrow grooves above periphery of last whorl) than in *N. aspera* (5–7 primary grooves on spire whorls, 7–10 wide grooves above periphery of last whorl). Anatomically, there is a small, but apparently consistent, difference in the penis; the filament

of *N. tenuistriata* bears annular wrinkles; it is also less obviously tapering than in *N. aspera* and the basal projection is often longer. The relative radular length is considerably greater in *N. aspera* (but this may be subject to variation according to rate of wear and requires further study). The paraspermatozoa are similar (although since most of the available specimens of *N. aspera* were immature, few sperm cells have been seen). Both species show a preference for exposed oceanic coasts. The similarities suggest a close relationship, and the differences could possibly be explained by geographical variation within a single species. However, a single sample has been seen from Corinto (Nicaragua; LACM 149776) which contains six examples of typical *N. aspera* and one shell tentatively identified as *N. tenuistriata*. This shell is atypical, lacking the dark spiral band and showing a slightly convex profile; nevertheless, the large size (16.7 mm), white ground color, and fine sculpture apparently preclude any other species. The resemblance is closest to some shells of *N. tenuistriata* from the nearest known localities to the south, in Costa Rica (Figure 11O). In any case, this shell (and its sympatric *N. aspera*) gives no suggestion of a merging of the shell characters of the two forms at this point of contact of their ranges. For these reasons, the two are believed to be distinct, although further sympatric records and genetic evidence are desirable to test this conclusion.

A commensal polyclad flatworm was found in the mantle cavity of one specimen (Punta Chocolatera, Guayas, Ecuador; BMNH), as also reported here for *N. modesta*, *N. conspersa*, and *N. apicina*.

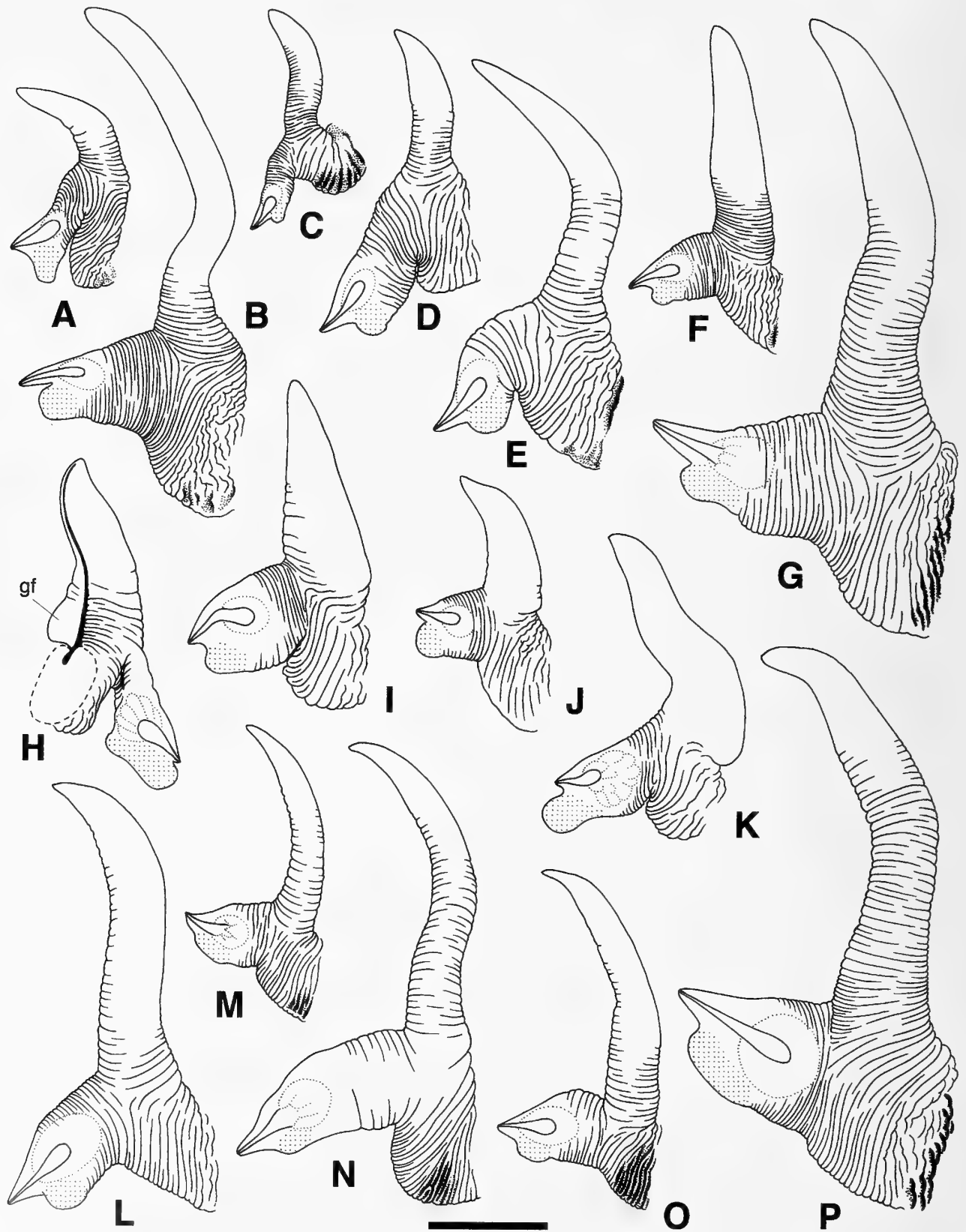
#### *Nodilittorina dubiosa* (C. B. Adams, 1852)

(Figures 9D, 11P–Z, 13M–Q, 15C, I, N, 17)

*Littorina aspera*—Philippi, 1847:2:200, *Littorina* pl. 4, fig. 13 (in part, includes *N. aspera*). Carpenter, 1857a:230, 326 (in part, includes *N. aspera*). Carpenter, 1864b:623 (in part, includes *N. aspera*). Weinkauff, 1882:60–61, pl. 8, figs. 2, 3 (in part, includes *N. aspera*). Weinkauff, 1883:220 (in part, includes *N. aspera*, *N. apicina*, *N. penicillata*).

*Littorina aspera*—C. B. Adams, 1852a:394–395 (in part, in-

Figure 13. Penes of *Nodilittorina aspera* group: *N. aspera* (A–F), *N. tenuistriata* Reid, sp. nov. (G–L), and *N. dubiosa* (M–Q). A, B. Puerto Marques, Acapulco, Guerrero, Mexico (BMNH 20001217; shell H = 10.6 mm, 11.3 mm). C, F. Cabo San Lucas, Baja California Sur, Mexico (BMNH 20001218, shell H = 8.9 mm, 10.2 mm). D, E. Puerto Angel, Oaxaca, Mexico (D, BMNH 20001213; shell H = 11.1 mm; E, BMNH 20001214, shell H = 12.7 mm). G, H. Playa de Manuel Antonio, Puerto Quepos, Costa Rica (BMNH 20001223; shell H = 12.9 mm, 10.5 mm). I. Muisne, Esmeraldas, Ecuador (BMNH 20001225; shell H = 11.7 mm). J, L. Punta Chocolatera, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20000314; shell H = 5.0 mm, 11.3 mm). K. Atacames, Esmeraldas, Ecuador (BMNH 20001221; shell H = 10.3 mm). M. Tarcoles, Costa Rica (BMNH 20001230; shell H = 10.5 mm). N. Paitilla Beach, Panama City, Panama (USNM 733196; shell H = 9.5 mm). O. Coco, W of Liberia, Costa Rica (BMNH 20001228; shell H = 3.9 mm). P, Q. Punta Morales, Golfo de Nicoya, Costa Rica (BMNH 20001229; shell H = 11.3 mm, 8.6 mm). Abbreviation: pgd, penial glandular disc (stipple). Shading conventions as in Figure 3. Scale bar = 1 mm.



- cludes *N. aspera*). C. B. Adams, 1852b:170–171 (in part, includes *N. aspera*). Carpenter, 1857a:186, 273 (not Philippi, 1846). Mørch, 1860:69 (not Philippi, 1846). Carpenter, 1863:352 (in part, includes *N. aspera*). Biolley, 1907:23 (not Philippi, 1846). Morrison, 1946:10 (not Philippi, 1846). Keen, 1958:282, fig. 173 left (in part, includes *N. aspera*). Hertlein, 1963:239 (as *aspersa*; not Philippi, 1846). Keen, 1971:365, fig. 181 (part) (in part, includes *N. aspera*, *N. penicillata*, *N. apicina*, *N. modesta*, *N. interrupta*). Montoya, 1983:332 (in part, includes *N. aspera*).
- Littorina (Melarhapha) aspera*—von Martens, 1900:577, 587, ? pl. 43, fig. 16 (in part, includes *N. aspera*, *N. apicina*).
- Littorina (Austrolittorina) aspera*—Abbott, 1974:69 (in part, includes *N. aspera*, *N. apicina*, *N. penicillata*).
- Nodilittorina (Nodilittorina) aspera*—Reid, 1989a:99, fig. 11k (egg capsule) (in part, includes *N. aspera*). Kaiser, 1997:27.
- Nodilittorina aspera*—Finet, 1994:18.
- Littorina parvula* ?—C. B. Adams, 1852a:398–399 (*Littorina parvula* Philippi, 1849, is a *nomen dubium*, see synonymy of *N. apicina*). C. B. Adams, 1852b:174–175.
- Littorina dubiosa* C. B. Adams, 1852a:398–399, 537 (Taboga [Taboga Island], Panama; lectotype (Turner, 1956: 118, pl. 13, fig. 13) MCZ 186573, seen, Figure 11Z herein; approx. 300 paralectotypes MCZ 139045, seen; 17 paralectotypes BMNH 1865.11.22.33, seen). C. B. Adams, 1852b:174–175, 313.
- Littorina* ? *parvula* var. *dubiosa*—Carpenter, 1857a:273.
- Littorina aspera dubiosa*—Turner, 1956:45–46, pl. 13, fig. 13.
- Littorina dubiosa dubiosa*—Keen, 1958:282, fig. 175.
- Littorina philippii*—Carpenter, 1863:352–353 (in part, includes *N. apicina*; not Carpenter, 1857 = *N. apicina*).

**Taxonomic history:** This species exhibits a range of shell sculpture from smooth to strongly ribbed. The ribbed shells have in the past been invariably identified as *aspera*, a name now restricted to a similar but largely allopatric species occurring mainly in Mexico (see also Taxonomic History of *N. aspera*). The fact that the species was named at all is owing to the rather common occurrence in Central America of a dwarf, smooth form that is superficially very different from larger ribbed shells. These smooth shells have frequently been confused with *N. apicina* (see Taxonomic History of that

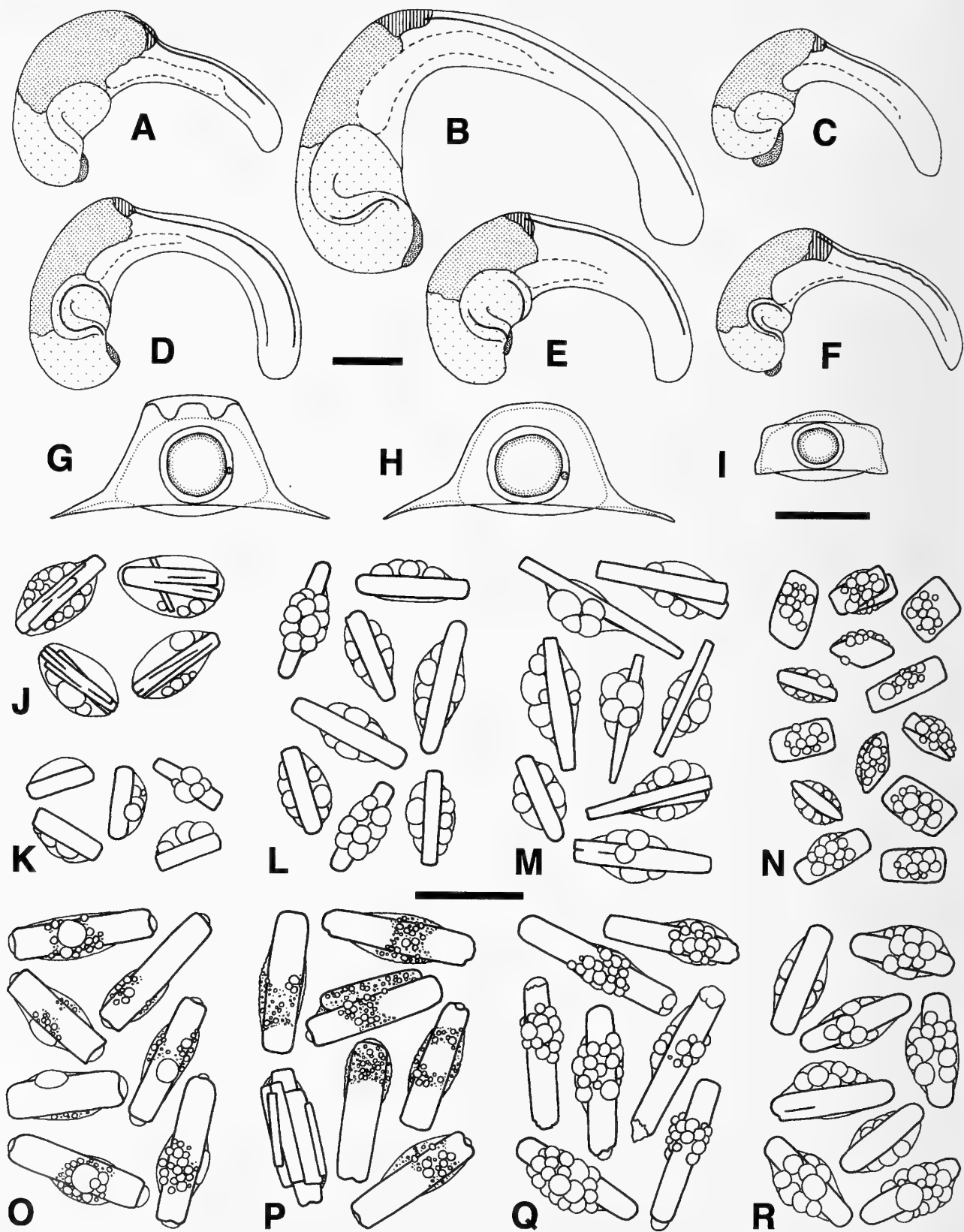
species). In fact, C. B. Adams (1852a) introduced his new species under the heading “*Littorina parvula*?” (here considered a *nomen dubium*, possibly a synonym of *N. apicina*) and prefaced the description with the qualification “If our shell is distinct from Philippi’s species, it may take the name of *L. dubiosa*. . .” (This conditional proposal does not prevent availability of the name; ICZN, 1999, Art. 11.5.1.) The shells on which he based his new taxon do indeed bear a superficial resemblance to *N. apicina*, but that species is rare in Panama and is not represented among the syntypes of *L. dubiosa*, or in the large collection of littorinids from Panama in the C. B. Adams Collection in the BMNH. Adams himself identified larger shells of *N. dubiosa* from Panama as *Littorina aspera*. Since its description, the name *dubiosa* has been used as valid only twice (Turner, 1956; Keen, 1958).

**Diagnosis:** Shell large or small, spire whorls slightly rounded, spire profile often slightly concave; 6–8 primary spiral grooves; 7–11 grooves above periphery of last whorl; sculpture of deep grooves up to 2 times rib width on last whorl of large shells, but grooves narrow or faint on small shells; fawn with oblique brown axial stripes and (on spire whorls only) a broad spiral zone of blue-grey just above periphery. Penis with stout, gradually tapering filament; large mamilliform gland (larger than small glandular disc) almost filling short projection of base.

**Material examined:** 55 lots (including 21 penes, 5 sperm samples, 5 pallial oviducts, 1 spawn sample, 4 radulae).

**Shell (Figures 11P–Z):** Mature shell height 3.9–19.4 mm. Shape high-turbinata (H/B = 1.32–1.77, SH = 1.57–1.92); spire whorls slightly rounded, suture distinct; spire profile sometimes slightly concave, giving slight onion shape; periphery of last whorl weakly angled. Columella concave, hollowed, and slightly pinched at base; small eroded parietal area. Sculpture of (5) 6–8 primary spiral grooves on spire whorls; ribs subequal, or slightly wider at suture and periphery; 7–11 grooves above periphery of last whorl, secondary sculpture usually absent, but rarely single narrow secondary riblets appear in grooves, in-

Figure 14. Penes of *Nodilittorina aspera* group (continued): *N. apicina* (A–G), *N. penicillata* (H–K), and *N. paytensis* (L–P). A. Bahía Santa María, Cabo San Lucas, Baja California Sur, Mexico (BMNH 20001232; shell H = 7.2 mm). B. Mazatlán, Sinaloa, Mexico (BMNH 20001241; shell H = 9.9 mm). C, D. Playa de los Muertos, Puerto Vallarta, Jalisco, Mexico (BMNH 20001242; shell H = 3.8 mm, 5.6 mm). E. Puerto Marques, Acapulco, Guerrero, Mexico (BMNH 20001237; shell H = 7.8 mm). F. Playa de Manuel Antonio, Puerto Quepos, Costa Rica (BMNH 20001243; shell H = 6.0 mm). G. Same, Esmeraldas, Ecuador (BMNH 20001236; shell H = 11.8 mm). H, K. Punta San Felipe, Baja California Norte, Mexico (BMNH 20001250; shell H = 8.2 mm, 7.7 mm). I. Topolobampo, Sinaloa, Mexico (BMNH 20001247; shell H = 10.6 mm). J. Cabo San Lucas, Baja California Sur, Mexico (BMNH 20001244; shell H = 7.6 mm). L. Ballenita, Guayas, Ecuador (BMNH 20001257; shell H = 9.5 mm). M. Anconcito, Guayas, Ecuador (BMNH 20001254; shell H = 5.3 mm). N. Atacames, Esmeraldas, Ecuador (BMNH 20001253; shell H = 10.0 mm). O. Playa de Manuel Antonio, Puerto Quepos, Costa Rica (BMNH 20001252; shell H = 8.0 mm). P. Same, Esmeraldas, Ecuador (BMNH 20001260; shell H = 10.9 mm). Abbreviation: gf, glandular flange. Shading conventions as in Figures 3, 13. Scale bar = 1 mm.



creasing number of grooves above periphery to 13–15; development of sculpture is variable; on last whorl of large shells grooves enlarge to 1–2 times width of intervening ribs, which become raised, rounded cords; sometimes only grooves just above and below periphery become enlarged, others remaining as incised lines; in dwarf forms all grooves may be faint, or grooves become obsolete, remaining only near periphery and suture; spiral microstriae absent. Protoconch not seen. Ground color cream to fawn, with narrow oblique brown stripes; typically, on lower half of spire whorls a broad blue-grey background zone (not a discrete dark band) which becomes indistinct on last whorl where (in corresponding region) brown pattern may appear as a fine tessellation; in dwarf and smooth shells ground color is white to cream, with broad blue-grey band just above periphery and a blue-grey line below periphery, usually with oblique or wavy axial stripes superimposed (although these may be faint or absent); aperture brown, external pattern showing through, with 2 pale spiral bands at base and shoulder; columella brown.

**Animal:** Head black, only rarely an unpigmented stripe across snout, tentacle pale at base and around eye, with two longitudinal black stripes and black spot at tip; sides of foot speckled black. Opercular ratio 0.37–0.41. Penis (Figures 13M–Q): filament moderately long, gradually tapering, smooth, thickened, and glandular, 0.6–0.8 total length; sperm groove open to tip; mamilliform gland larger than glandular disc, often swollen and almost filling the short projection of base; penis unpigmented or only slightly pigmented at base. Euspermatozoa 64  $\mu\text{m}$ ; paraspermatozoa (Figure 15N) with single (rarely double) broad rectangular or trapezoidal rod-pieces, 11–16  $\mu\text{m}$  filling cell, with few large round granules. Pallial oviduct (Figure 15C) with large copulatory bursa opening at half length of straight section and extending back to albumen gland. Spawn (Figure 15I) a simple biconvex capsule 140  $\mu\text{m}$  diameter, with upper ring and slight lower flange, containing single ovum 40  $\mu\text{m}$  diameter.

**Radula (Figure 9D):** Relative radular length 5.6–7.8.

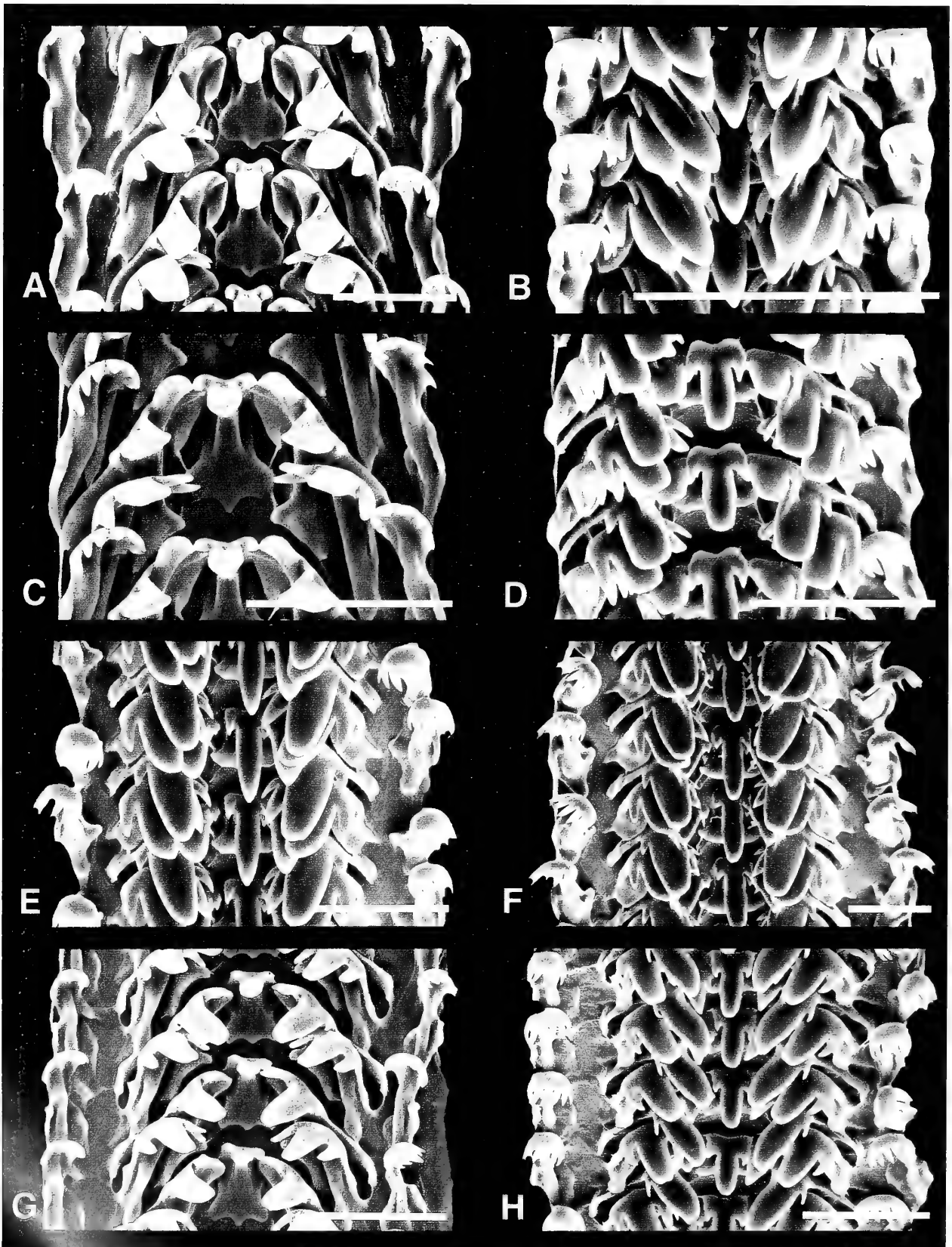
Rachidian: length/width 1.36–1.90; major cusp elongate, rounded at tip. Lateral and inner marginal: major cusps elongate, rounded at tip. Outer marginal: 7–9 cusps.

**Habitat:** Abundant on moderately exposed to sheltered shores; found even in somewhat turbid embayments and close to stream outflows; therefore shows a continental distribution pattern on the mainland. However, the species also occurs on Isla del Coco and there is a single record from the Galápagos. Occurs on rocks (including volcanic conglomerate, tuff, sandstone) in littoral fringe and at top of eulittoral zone. Dwarf specimens found in pools at top of shore. Rare on exposed coasts, where small specimens nestle among barnacles and in crevices. Microsympatric with *N. apicina* on moderately exposed shores, but extends to higher levels. On sheltered shores (particularly in Gulf of Panama) *N. dubiosa* is often the only member of the *N. aspera* group to be found. Ecological studies of “*Littorina aspera*” in Panama by Markel (1971), Garrity & Levings (1981), and Garrity (1984) were based largely on this species, but may have included others in the *N. aspera* complex.

**Range (Figure 17):** El Salvador (and perhaps Guatemala) to Colombia, Isla del Coco, Galápagos Islands. Range limits: Guatemala (LACM 149773, 1 specimen); Punta Amapala, El Salvador (USNM 780445, 10 specimens); San Juan del Sur, Nicaragua (USNM 23304); Punta San Francisco, Bahía Solano, Colombia (USNM 819739); Ladrilleros, Colombia (USNM 807724); Isla Gorgona and Isla Gorgonilla, Colombia (USNM 819729, 3 specimens); Isla del Coco, Costa Rica (KLK; 2 collections, 15 specimens); Punta Pitt, Isla San Cristobal, Galápagos (BMNH 20001227, 4 specimens). The species is rare in the Galápagos Islands, with only a single verified collection; records in faunal lists from the islands (Schwengel, 1938; Finet, 1985, 1994; Kaiser, 1993, 1997) are based on likely misidentification and on literature records; the two lots in LACM quoted by Finet (1994) are *N. conspersa* and an incorrectly localized sample of a mixture of *N. aspera* and *N. penicillata*. The unlocalized record from Guatemala is at least 150 years old and requires verification,

Figure 15. Pallial oviducts (A–F), egg capsules (G–I), and paraspermatozoa (J–R) of *Nodilittorina aspera* group: *N. aspera* (A, J, K), *N. tenuistriata* Reid, sp. nov. (B, L, M), *N. dubiosa* (C, I, N), *N. apicina* (D, O, P), *N. penicillata* (E, Q), *N. paytensis* (F–H, R). A, J. Mazatlán, Sinaloa, Mexico (BMNH 20001219; shell H = 11.0 mm). B. Same, Esmeraldas, Ecuador (BMNH 20001226; shell H = 14.2 mm). C. Punta Amapala, El Salvador (USNM 332140; shell H = 10.2 mm). D. Puerto Marques, Acapulco, Guerrero, Mexico (BMNH 20001237; shell H = 11.5 mm). E, Q. Punta San Felipe, Baja California Norte, Mexico (BMNH 20001250; shell H = 10.5 mm). F. Punta Chocolatera, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20001259; shell H = 10.8 mm). G, H. Ballenita, Guayas, Ecuador (BMNH 20001257). I, N. Punta Morales, Golfo de Nicoya, Costa Rica (BMNH 20001229). K. Puerto Marques, Acapulco, Guerrero, Mexico (BMNH 20001217). L. Atacames, Esmeraldas, Ecuador (BMNH 20001221). M. Punta Chocolatera, Peninsula Santa Elena, Guayas, Ecuador (BMNH 2000314). O. Playa de los Muertos, Puerto Vallarta, Jalisco, Mexico (BMNH 20001242). P. Same, Esmeraldas, Ecuador (BMNH 20001236). R. Same, Esmeraldas, Ecuador (BMNH 20001260). Shading conventions as in Figure 4. Scale bars: A–F = 1 mm; G–I = 0.1 mm; J–R = 20  $\mu\text{m}$ .





but would be of interest since it lies in the Central American Gap (see Discussion).

**Remarks:** This species displays a confusing variation in shell size, coloration, and sculpture. As a result, the small, smooth, or weakly sculptured examples have in the past been classified with *N. apicina*, whereas the large, striped, strongly ribbed shells have been included with *N. aspera*. As in some other eastern Pacific *Nodilittorina* species, this shell variation is correlated with microhabitat, the dwarf forms (Figures 11R, S, Z) occurring in high-level saline pools and on exposed coasts, and the larger shells on more sheltered shores where conditions for growth are presumably more favorable. Unlike *N. aspera* and *N. tenuistriata*, the dwarf forms are not darkly patterned.

On moderately exposed shores (for example in Costa Rica) *N. dubiosa* is frequently sympatric with *N. apicina*, and discrimination between them is sometimes difficult. In the latter, the sculpture is of incised lines only (never wide grooves), the color paler (white ground color, often with a zigzag or finely tessellated pattern), the columella long and straight, and the overall shape more oblique. In contrast, when present, the grooves on the shell of *N. dubiosa* become wide at least near the periphery of the last whorl (or over the whole whorl), the ground color is cream to fawn (usually with a regular lined pattern), and the columella slightly curved. Identification of males can be confirmed by the penis, which in *N. apicina* has a long, wrinkled filament and protruberent glandular disc, and in *N. dubiosa* a smooth, tapering filament and much larger mamilliform gland. The form of the paraspermatozoa also differs.

Larger examples of *N. dubiosa* share with the more northern species, *N. aspera*, the unusual feature of wide grooves (up to twice the width of the intervening raised ribs) on the last whorl, besides a tendency to an onion-shaped profile, and for these reasons the species was at first believed to be simply a southern form of *N. aspera*. However, there is a difference in the penis, the mamilliform gland of *N. dubiosa* being much larger, almost filling the basal projection. Unfortunately, only two samples of sperm were available from *N. aspera*, but in these the long rod-pieces differed from the broader or trapezoidal rod-pieces in five samples from *N. dubiosa*. Close examination of the shells reveals that their color patterns are distinct, *N. aspera* showing coarser axial stripes, white ground color, and a strong dark spiral band above

the periphery of at least the spire whorls, in contrast to the cream or fawn ground color with diffuse peripheral blue-grey band, and finer axial stripes of *N. dubiosa*. The typical habitats of the two are also suggestive, on exposed coasts in *N. aspera*, and mainly on moderately sheltered shores in *N. dubiosa*. For these reasons, the two are here regarded as separate species. Their known distributions overlap slightly, in El Salvador and Nicaragua, but they have not yet been recorded at the same locality in order to confirm that their characters remain distinct. Additional, if indirect, evidence for their separate status is that there is a third species, *N. tenuistriata*, which has been found in sympatry with both *N. aspera* and *N. dubiosa*, and is itself the more likely sister-species of *N. aspera*. *Nodilittorina tenuistriata* is easily separated from *N. dubiosa* by its finer sculpture (7–10 primary spiral grooves on spire whorls, 10–15 narrow grooves above periphery of last whorl), broad dark spiral band above the periphery, and more rounded whorls; it also occurs on more exposed shores than *N. dubiosa*.

Since the typical habitat on relatively sheltered shores and tolerance of turbidity and freshwater influence suggest a continental distribution, the records of *N. dubiosa* on Isla del Coco and the Galápagos are unexpected. The provenance of these specimens is completely reliable. The specimens from Isla del Coco are dry shells, but these are entirely typical (Figure 11W). The four from the Galápagos are elongate and weakly sculptured, with an unusually prominent dark band above the periphery (Figure 11Q); the two dissected specimens were female so that the important characters of the penis were not available. The shape of the columella shows that these are not *N. apicina*, and the weak sculpture and brownish coloration suggest that they are not *N. tenuistriata*. The occurrence of *N. dubiosa* on Isla del Coco and the Galápagos Islands could be related to strong currents that originate in the Gulf of Panama from February to April and flow toward the southwest (Finet, 1991).

#### *Nodilittorina apicina* (Menke, 1851)

(Figures 12A–I, 14A–G, 15D, O, P, 16A–D, 17)

? *Littorina parvula* Philippi, 1849:149 (Panama; types lost; *nomen dubium*).

*Littorina* [*aspera* var.] *apicina* Menke, 1851:164 (Mazatlan [Mexico]; lectotype (here designated), 7.1 × 4.8 mm)

Figure 16. Radulae of *Nodilittorina apicina* (A–D), *N. paytensis* (E), *N. peruviana* (F), and *N. araucana* (G, H). A, B. Playa de los Muertos, Puerto Vallarta, Jalisco, Mexico (BMNH 20001242; flat, shell H = 8.7 mm; at 45°; shell H = 5.0 mm). C, D. Playa de Manuel Antonio, Puerto Quepos, Costa Rica (BMNH 20001243; two views of radula, flat and at 45°; shell H = 7.1 mm). E. Punta Chocolatera, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20001259; at 45°; shell H = 9.1 mm). F. Playa la Lisera, Arica, Tarapacá, Chile (BMNH 20001271; at 45°; shell H = 12.1 mm). G, H. Las Cruces, Valparaíso, Chile (BMNH 20001263; two views of radula, flat and at 45°; shell H = 7.8 mm). Scale bars = 50 µm.

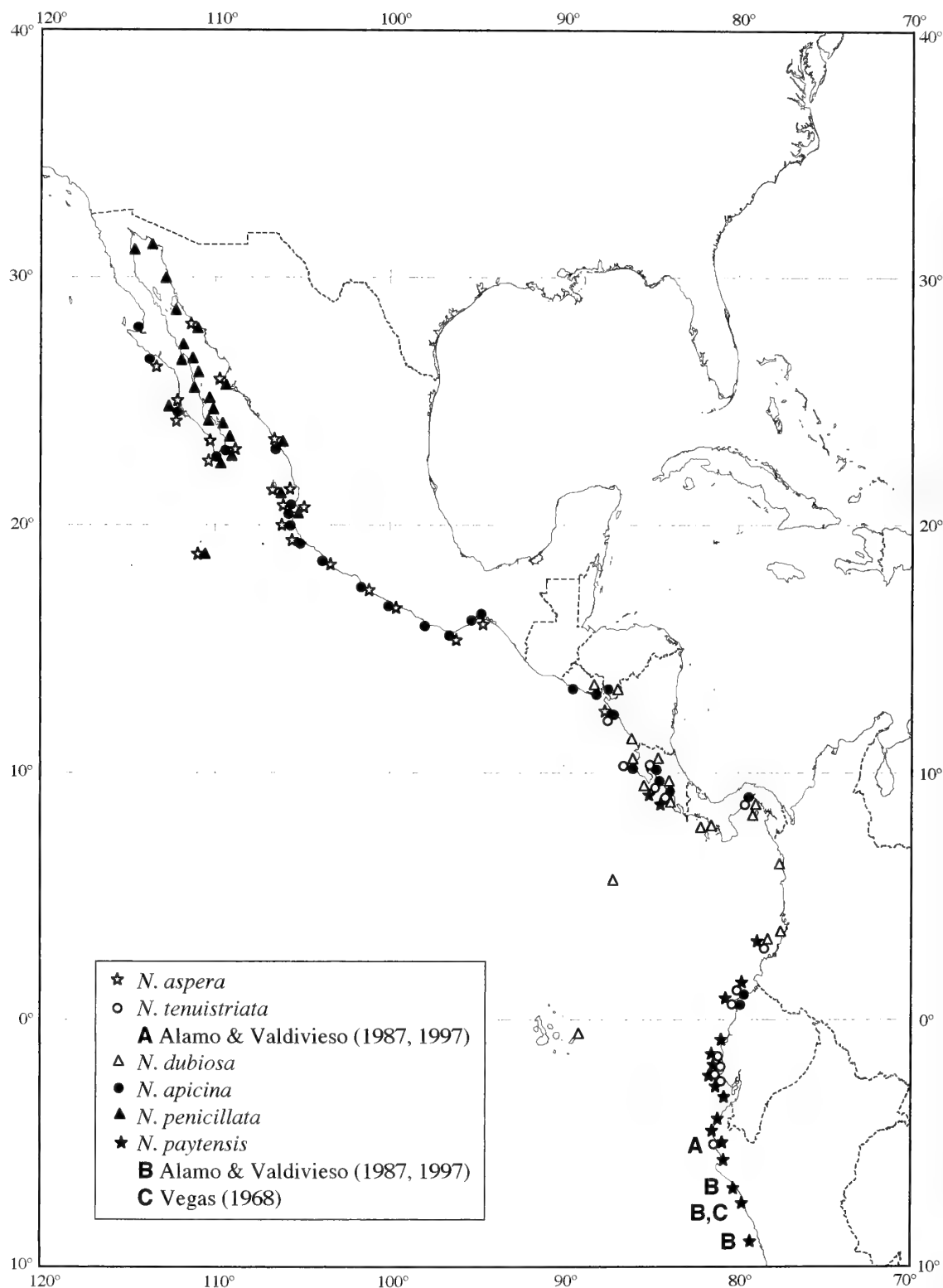


Figure 17. Geographical distribution of species of the *Nodilittorina aspera* group (records based on material examined and quoted literature sources). There are in addition an unlocalized record of *N. aspera* from El Salvador (von Martens, 1900) and another of *N. dubiosa* from Guatemala (LACM 149773).

SMF 314713a, Figure 12F; 3 paralectotypes SMF 314713/3; types seen).

*Litorina (Melaraphe) philippii* Carpenter, 1857b:349–350 (Mazatlan [Mexico]; lectotype (here designated, 10.9 × 7.3 mm) BMNH 1857.6.4.1682/1, Figure 12B; 109 paralectotypes BMNH 1857.6.4.1671–1685 (100 shells and 9 separate opercula; one shell figured by Keen, 1968, pl. 57, fig. 54; one shell is *N. aspera*); types seen).

*Litorina philippii*—Carpenter, 1857a:257, 326, 364. Carpenter, 1864b:546, 550, 623 (in part, includes *N. penicillata*, *N. dubiosa*). Weinkauff, 1882:104 (in part, includes *N. penicillata*).

*Littorina (Melaraphe) aspera* var. *philippii*—Tryon, 1887:249, pl. 44, fig. 84.

*Littorina (Melaraphe) philippii*—von Martens, 1900:577, 584, pl. 43, fig. 12 (in part, includes *N. dubiosa*, *N. penicillata*, *N. modesta*).

*Littorina philippii*—Carpenter, 1863:352–353 (in part, includes *N. dubiosa*). Pilsbry & Lowe, 1932:124.

*Littorina dubiosa philippii*—Keen, 1958:282, 623, fig. 175b. Keen, 1968:410, pl. 57, fig. 54.

*Littorina aspera*—Weinkauff, 1883:220 (in part, includes *N. aspera*, *N. dubiosa*, *N. penicillata*).

*Littorina aspera*—Keen, 1971:365, fig. 181 (part) (in part, includes *N. aspera*, *N. penicillata*, *N. dubiosa*, *N. modesta*, *N. interrupta*).

*Littorina (Austrolittorina) aspera*—Abbott, 1974:69 (in part, includes *N. aspera*, *N. dubiosa*, *N. penicillata*).

*Littorina (Melaraphe) philippii* var. *subsuturalis* von Martens, 1900:577, 585, pl. 43, fig. 17 (San José, W. Guatemala; five syntypes MNB, seen).

**Taxonomic history:** *Litorina parvula* was only briefly described, and not figured, by Philippi (1849), and no types have been traced. The color pattern of undulating black lines, impressed spiral lines (7 on last whorl) and small size (7.6 × 4.9 mm) suggest the present species, but do not exclude others that are similar. The material was collected by the author's brother, E. B. Philippi, from "Panama," but he visited both the eastern Pacific Ocean and Caribbean Sea, so the description might even apply to one of the several black and white *Nodilittorina* species of the Caribbean. This is regarded as a *nomen dubium*. The type material of *Litorina apicina*, recently rediscovered in the Bronn collection in SMF, confirms the precise description given by Menke (1851). The name was proposed preliminarily, within his account of *Litorina aspera*. (Translation from the German: "Without separating this form presently as a distinct species, I call it preliminarily *L. apicina*." This is here interpreted as introduction of an infraspecific name and not as publication in synonymy, which would invalidate the name for it has not been used since; ICZN, 1999, Art. 11.6.) Menke (1851) applied the name to small shells (7.6 × 4.8 mm) that he separated from the young of *Litorina aspera* by the more tumid shape, lack of dark spiral band, and evenly spaced ribs separated by narrow grooves; these characters are precisely those that separate this species from *N. aspera*, which is the only other black and white littorinid common at Mazatlán. In the Reigen Collection, Car-

penter (1857b) had a large amount of material also from Mazatlán and he recognized that this species was distinct from *N. aspera* at the same locality. Nevertheless, he rejected Menke's name, arguing that it applied to young *aspera* and was in any case inappropriate for a species which was almost always eroded; on these dubious grounds he introduced *Litorina philippii*. Carpenter's (1857b) species is represented by 101 shells from the Reigen Collection in BMNH, of which only one is *N. aspera*, and a lectotype is here selected. The choice of *apicina* rather than *philippii* as the valid name for this species is in accordance with the principle of priority; although the younger name has been used more frequently in the literature, it has not been used sufficiently often for precedence to be reversed (ICZN, 1999, Art. 23.9) and not at all since 1968. Three of the five syntypes of *L. philippii* var. *subsuturalis* have an unusually dark peripheral band, but the form of the columella and of the spiral sculpture confirms that all belong to *N. apicina* (and not to *N. dubiosa*).

A number of authors (Carpenter, 1857a, b, 1863, 1864b; Weinkauff, 1882; von Martens, 1900; Pilsbry & Lowe, 1932; Keen, 1958) have discriminated a smaller, smooth-shelled species from the larger, more strongly sculptured shells named *aspera*. The names *parvula*, *apicina*, *philippii*, and *dubiosa* have been applied to this smaller species, but the grouping is often not a natural one since most species in the *N. aspera* group can develop a relatively smooth shell. In some geographical areas, a distinction based on sculpture alone does indeed correctly separate sympatric species. Thus, at Mazatlán, the large, ribbed shells are *N. aspera* s.s., while small, smoother ones are *N. apicina* (the only other member of the *N. aspera* group to occur there, *N. penicillata*, is rare), as correctly recognized by both Menke (1851) and Carpenter (1857a, b; as *philippii*). Elsewhere, the smooth-shelled forms include *N. penicillata* and weakly sculptured examples of *N. dubiosa*, which have therefore often been included with the present species (Carpenter, 1863, 1864b; Weinkauff, 1882; von Martens, 1900; Keen, 1958). Weinkauff (1883) first synonymized these smooth species with *aspera*, as also done by Keen (1971) and Abbott (1974), and *N. apicina* has not since been recognized as distinct.

**Diagnosis:** Shell of moderate size, spire whorls moderately rounded, spire profile distinctly convex; columella long, straight; 5–7 primary spiral grooves; 9–12 grooves above periphery of last whorl; sculpture of incised lines or narrow grooves; white with grey-brown to black oblique or zigzag axial lines; no dark spiral bands, but often an indistinct greyish zone from periphery to shoulder, where pattern tends to form tessellation rather than axial lines. Penis with long, wrinkled filament; mamilliform gland and protruberant glandular disc on projection of base.

**Material examined:** 56 lots (including 32 penes, 9 sperm samples, 4 pallial oviducts, 6 radulae).

**Shell (Figures 12A–I):** Mature shell height 2.9–14.9 mm. Shape obliquely high-turbinate to moderately tall ( $H/B = 1.33\text{--}1.71$ ,  $SH = 1.31\text{--}1.94$ ); spire whorls moderately rounded, suture distinct; spire profile usually convex, giving oblique shape; periphery of last whorl weakly angled. Columella long, straight, slightly hollowed, and pinched at base; small eroded parietal area. Sculpture of 5–7 primary spiral grooves on spire whorls; these remain as incised lines, or become wider toward periphery of last whorl (occasionally up to half width of peripheral rib), only rarely become obsolete; grooves number 9–12 above periphery of last whorl, or occasionally up to 15 if some secondary grooves appear; ribs may be subequal, but usually 3–4 posterior ribs are slightly wider, and peripheral rib is slightly larger and raised; spiral microstriae absent. Protoconch not seen. Color white, with variable dark pattern; commonly brown oblique or zigzag axial lines, often forming grey-brown tessellation in greyish zone from periphery halfway to suture; sometimes strong black oblique axial stripes, occasionally anastomosing in peripheral zone to give irregular black band (dwarf shells from exposed localities only); sometimes a fine pallid beige or brown tessellation throughout, darkening to grey in zone from periphery halfway to suture; this indistinct greyish zone with darker pattern is typical, but distinct dark spiral lines or bands are almost always absent (rarely, an anterior spiral band is present on upper spire whorls only); aperture brown, with pattern showing through and 2 pale spiral bands, at base and shoulder; columella brown.

**Animal:** Head black, sometimes a thin unpigmented stripe across snout, tentacle pale at base and around eye, with two longitudinal black lines, sometimes fusing distally or with black spot at tip; sides of foot grey to black. Opercular ratio 0.31–0.41. Penis (Figures 14A–G): filament long, gradually tapering, rounded at tip, lower half of filament with annular wrinkles and not clearly differentiated from wrinkled penial base, filament about 0.5–0.7 total length; sperm groove open to tip; mamilliform gland and large glandular disc on projection of base, disc protrudes as a lobe; penis unpigmented or only slightly pigmented at base. Euspermatozoa 54–68  $\mu\text{m}$ ; paraspermatozoa (Figures 15O, P) of single, long, stout, parallel-sided rod-pieces, 21–32  $\mu\text{m}$ , often with rounded or pointed terminal caps, attached cytoplasm usually thin, with small indistinct granules, often one large granule (perhaps the nucleus) visible. Pallial oviduct (Figure 15D) with large copulatory bursa opening at three-quarters of the length of straight section and extending back to albumen gland. Spawn not observed.

**Radula (Figures 16A–D):** Relative radular length 2.8–4.8. Rachidian: length/width 1.42–2.0; major cusp elongate, rounded, or pointed at tip. Lateral and inner marginal: major cusps elongate; rounded, blunt or pointed at tip. Outer marginal: 6–8 cusps.

**Habitat:** Clustered in crevices and at margins of pools, in upper eulittoral and lower littoral fringe; sometimes among barnacles and mussels in mid-eulittoral zone; exposed and moderately sheltered coasts; recorded on granite, basalt, sandstone, mudstone, and concrete; usually common to abundant. Although for most of its range this species is found only in relatively exposed, oceanic situations, there are no records from offshore islands and it is sometimes found in embayments and lagoons (e.g., Laguna Ojo de Liebre and Ventosa Bay, Mexico; Golfo de Nicoya, Costa Rica). Overlapping with, but zoned slightly below *N. dubiosa* and *N. aspera* in Central America and Mexico, respectively; in Ecuador microsympatric with *N. paytensis*, but below *N. tenuistriata*.

**Range (Figure 17):** Western Baja California to northern Ecuador. Range limits: Laguna Ojo de Liebre, Baja California Sur (USNM 130598, 2 specimens); Punta Abreojos, Baja California Sur (USNM 862105); Bahía Magdalena, Baja California Sur (USNM 264566); Cabo San Lucas, Baja California Sur (BMNH 20001231); Bahía Santa María, E of Cabo San Lucas, Baja California Sur (BMNH 20001232); Mazatlán, Sinaloa (BMNH 20001233); Bahía Ventosa, Golfo de Tehuantepec, Oaxaca (USNM 60449); Salina Cruz, Oaxaca (LACM 149771); La Libertad, El Salvador (LACM 149774, 30 specimens); Punta Amapala, El Salvador (USNM 780445); Coyolita, Golfo de Fonseca, Honduras (USNM 749642); Corinto, Nicaragua (CAS 122359); Fort Amador, Panama (USNM 732997, 11 specimens; BMNH 20001234, 1 specimen); Atacames, Esmeraldas, Ecuador (BMNH 20001235); Punta Galera, Esmeraldas, Ecuador (USNM 711369). The species is common throughout most of its range, but is rare in the Gulf of Panama (only two records), perhaps connected with eutrophic and largely sheltered conditions there.

**Remarks:** Shape and sculpture are relatively constant in this species, but it displays some geographical variation in color pattern. In specimens from Mexico, the pattern is usually of dark axial stripes on a white ground, whereas in Central America and Ecuador the coloration is greyish or pallid, with a finer and more diffuse tessellation. In the former group, the most pronounced black and white striped pattern is often found in dwarf shells (Figure 12G) from shallow pools in the upper eulittoral zone; abrupt transitions to a paler and more tessellated pattern can sometimes be seen, suggesting phenotypic plasticity in the expression of coloration. At two localities (Puerto Vallarta, Jalisco; Puerto Quepos, Costa Rica), a commensal flatworm occurs occasionally in the mantle cavity (see Remarks on *N. modesta* group).

This species has the widest geographical range of all

the eastern Pacific *Nodilittorina* species and is the only one found both in central Mexico and south of Costa Rica. Over its range it is sympatric with several other species, and confusion is most likely with *N. aspera* and *N. penicillata* in Mexico, and with *N. dubiosa*, *N. paytensis*, and *N. tenuistriata* in Central and South America. Its most characteristic features are the relatively long and straight columella, the somewhat oblique shape (slightly *Succinea*-like), the relatively narrow grooves (not more than half width of ribs, distinguishing it from the more strongly sculptured examples of *N. aspera* and *N. dubiosa*), and the lack of a strong dark spiral band or line above the periphery (as present in *N. penicillata*, *N. aspera*, *N. tenuistriata* and usually in *N. paytensis*). Similarities of shell, paraspermatozoa, and geographical proximity suggest that its closest relative may be *N. penicillata*.

*Nodilittorina penicillata* (Carpenter, 1864)

(Figures 9H, 12J–Q, 14H–K, 15E, Q, 17)

*Littorina* (*philippii*, var.) *penicillata* Carpenter, 1864a:477 (Cape St Lucas [Cabo San Lucas, Baja California], Mexico; lectotype (here designated, 8.6 × 5.3 mm) USNM 4058, seen, Palmer, 1963:pl. 61, fig. 7, Figure 12L herein; 2 paralectotypes USNM 678691; 31 paralectotypes USNM 862110 (3 of these are *N. aspera*); 20 paralectotypes MCZ 086976; 1 paralectotype ANSP 212152; 26 paralectotypes BMNH 1865.11.6.70; 19 paralectotypes BMNH 19991559; all paralectotypes seen).

*Littorina philippii* var. *penicillata*—Weinkauff, 1882:104.

*Littorina* (*Melaraphe*) *aspera* var. *penicillata*—Tryon, 1887:250, pl. 44, fig. 85.

*Littorina* (*Melaraphe*) *philippii* var. *penicillata*—von Martens, 1900:577, 584–585, pl. 43, fig. 14.

*Littorina penicillata*—Pilsbry & Lowe, 1932:124.

*Littorina dubiosa penicillata*—Keen, 1958:282, fig. 175a. Palmer, 1963:334–335, pl. 61, fig. 7.

*Littorina* (*Austrolittorina*) *penicillata*—Rosewater, 1970:423.

*Nodilittorina* (*Nodilittorina*) *penicillata*—Reid, 1989a:99 (doubtfully included in synonymy of *N. aspera*).

*Littorina aspera*—Weinkauff, 1883:220 (in part, includes *N. aspera*, *N. dubiosa*, *N. apicina*).

*Littorina aspera*—Keen, 1971:365, fig. 181 (part) (in part, includes *N. apicina*, *N. aspera*, *N. dubiosa*, *N. modesta*, *N. interrupta*).

*Littorina* (*Austrolittorina*) *aspera*—Abbott, 1974:69 (in part, includes *N. aspera*, *N. dubiosa*, *N. apicina*).

**Taxonomic history:** This species was first described by Carpenter (1864a) as a variety of *L. philippii* (= *N. apicina*), characterized by its distinctive color pattern. It is represented by a large type series, of which the specimen figured by Palmer (1963) is here selected as the lectotype. Of the 79 known paralectotypes three are *N. aspera*, perhaps included inadvertently. Subsequent authors initially accepted this status as a color variety of a supposed smooth-shelled species named *philippii* or *dubiosa* (Weinkauff, 1882; von Martens, 1900; Keen, 1958; Palmer,

1963). Others synonymized it with *aspera*, which was believed to be a single widely variable species (Weinkauff, 1883; Tryon, 1887; Keen, 1971; Abbott, 1974). However, several authors have listed it as a distinct species; though the evidence has not been discussed, they were presumably impressed by the restriction of the distinctive color form to the Gulf of California and its sympatric occurrence with typical *N. aspera* (Pilsbry & Lowe, 1932; Rosewater, 1970; doubtful status in Reid, 1989a).

**Diagnosis:** Shell of moderate size, spire whorls moderately rounded; 6–9 primary spiral grooves; 8–11 grooves above periphery of last whorl; sculpture of incised lines only; white with oblique or waved grey to brown axial lines, one spiral dark line above periphery and one below. Penis with broad, pointed filament, thickened at base; mamilliform gland and large glandular disc on projection of base.

**Material examined:** 57 lots (including 15 penes, 2 sperm samples, 4 pallial oviducts, 4 radulae).

**Shell (Figures 12J–Q):** Mature shell height 3.9–15.3 mm. Shape moderately tall (H/B = 1.48–1.84, SH = 1.60–2.05); spire whorls moderately rounded, suture distinct; spire profile straight to slightly convex; periphery of last whorl weakly angled. Columella concave, hollowed and pinched at base; no eroded parietal area. Sculpture of 6–9 primary spiral grooves on spire whorls; these remain as incised lines, numbering 8–11 above periphery of last whorl, or occasionally become faint or obsolete on shoulder; rarely (only on largest shells) a secondary groove divides each primary rib; ribs subequal, except peripheral rib which is twice as wide; spiral microstriae absent. Protoconch not seen. Color bluish white, with brown, grey, or black pattern of fine wavy or oblique axial lines; two narrow spiral lines of blue-grey or black, one on third rib above peripheral rib, one in groove immediately below peripheral rib, occasionally other dark lines appear in grooves between these prominent lines, or sometimes spiral lines may become obsolete on last whorl (spiral line above periphery remains visible on spire); aperture dark chestnut brown with 2 pale spiral bands, at base and shoulder, where external pattern shows through; columella blackish brown.

**Animal:** Head dark grey to black, sometimes a thin unpigmented stripe across snout, tentacle pale at base and around eye, with two longitudinal black lines and black spot at tip; sides of foot grey to black. Opercular ratio 0.41–0.51. Penis (Figures 14H–K): filament broad, pointed at tip, with thickened flange at base on medial side, smooth and clearly differentiated from wrinkled penial base, about 0.6–0.8 total length; sperm groove open to tip; mamilliform gland and large glandular disc on stout projection of base; penis unpigmented. Euspermatozoa 57–64 µm; paraspermatozoa (Figure 15Q) of single, long, stout, parallel-sided rod-pieces, 24–35 µm, with cluster



of large spherical granules attached. Pallial oviduct (Figure 15E) with large copulatory bursa opening halfway along straight section and extending back to albumen gland. Spawn not observed.

**Radula (Figure 9H):** Relative radular length 3.9–8.5. Rachidian: length/width 1.38–1.67; major cusp elongate, pointed to rounded at tip. Lateral and inner marginal: major cusps elongate, rounded at tip. Outer marginal: 8–9 cusps.

**Habitat:** Clustered in crevices and at margins of saline pools, in littoral fringe; sheltered and moderately exposed coasts; recorded on granite, conglomerate, basalt, and muddy rocks; usually common to abundant. Sympatric with *N. aspera* and *N. apicina* in southernmost Baja California, but extends to higher levels on the shore.

**Range (Figure 17):** Gulf of California, southern Baja California, to Mazatlán, Islas Tres Marías, Puerto Vallarta, and Islas Revillagigedo. Range limits: Bahía Magdalena, Baja California Sur (USNM 819877, 3 specimens); Cabo San Lucas, Baja California Sur (BMNH 2001244); Bahía Coyote, Bahía Concepción, Baja California Sur (USNM 558627, 1 specimen); San Felipe, Baja California Norte (BMNH 20001245); Puerto Peñasco, Sonora (USNM 701417); Puerto Libertad, Sonora (USNM 809245, 1 specimen); S end Isla del Tiburón, Sonora (USNM 264910, 6 specimens); San Carlos, 20 km W of Guaymas, Sonora (BMNH 20001246); Topolobampo, Sinaloa (BMNH 20001247); Mazatlán, Sinaloa (BMNH 1857.6.4.1670, 3 specimens; BMNH 20001248, 2 specimens); Isla María Cleofas, Islas Tres Marías (KLK, 1 specimen); Playa de los Muertos, Puerto Vallarta, Jalisco (BMNH 20001249, 1 specimen); Isla Socorro, Islas Revillagigedo (KLK, 2 specimens). This species has a curious distribution, being abundant in most parts of the Gulf of California (from Cabo San Lucas northward, including San Felipe, Guaymas, and Topolobampo), with the apparent exception of the area between Bahía Concepción and San Felipe (this gap is probably real, since the smaller species *N. albicarinata* is well represented by collections from this part of the Gulf). Only nine specimens have been seen from localities south of the Gulf of California, where it is a rare immigrant (von Martens, 1900, noted its rarity at Mazatlán).

**Remarks:** The shell characters are relatively constant in this species, which is most easily recognized by the pattern of fine “pencilled” axial and two spiral lines. In other species with dark spirals, a wider band is present, occupying the lower half of each spire whorl and a zone just above the periphery on the last whorl. This species most resembles *N. apicina*, and the two can be found commonly together in the vicinity of Cabo San Lucas (southernmost Baja California) and occasionally on the Mexican mainland. They can always be distinguished by color pattern, which in *N. apicina* from these areas is of

strong axial stripes with no spiral lines. These two are likely sister species, as suggested by similarity of shell sculpture, paraspermatozoa, and parapatric geographical distribution.

*Nodilittorina paytensis* (Philippi, 1847)

(Figures 12R–BB, 14L–P, 15F–H, R, 16E, 17)

- Littorina paytensis* Philippi, 1847:2:166, *Littorina* pl. 3, fig. 25 (Payta in Peruvia [Paita, Piura, Peru]; types presumed lost; lectotype (here designated) Philippi, 1847: *Littorina* pl. 3, fig. 25, central shell, Figure 12BB herein). Weinkauff, 1882:68, pl. 9, figs. 1, 4. Weinkauff, 1883:218.
- Littorina (Melaraphe) paytensis*—H. & A. Adams, 1854: 314. Tryon, 1887:250, pl. 45, figs. 90, 91 (in part, includes *N. araucana*).
- Littorina paytensis*—Vegas, 1968:8. Keen, 1971:366, fig. 184 (possible subspecies of *L. modesta*). Peña, 1971b: 47, pl. 1, fig. 5. Finet, 1985:13 (possible subspecies of *L. modesta*). Finet, 1994:18, 127 (possible synonym of *N. araucana*).
- Littorina (Littorinopsis) paytensis*—Rosewater, 1970:423. Alamo & Valdivieso, 1987:25, fig. 37. Alamo & Valdivieso, 1997:17, fig. 37. Paredes et al., 1999:22.
- Littorina modesta paytensis*—Vermeij, 1973:324.
- Nodilittorina paytensis*—Bandel & Kadolsky, 1982:3.
- Nodilittorina (Nodilittorina) paytensis*—Reid, 1989a:99 (doubtfully included in synonymy of *N. araucana*).
- Littorina araucana*—Dall, 1909:231, 285 (in part, includes *N. araucana*). Hertlein & Strong, 1955b:273–274 (in part, includes *N. araucana*).

**Taxonomic history:** Since it is found mainly in Colombia, Ecuador and northern Peru, where the malacological fauna has been poorly studied, this species has seldom appeared in the literature. With limited availability of specimens, some authors of revisions and lists have maintained it as a distinct species (Weinkauff, 1882, 1883; Rosewater, 1970; Keen, 1971) and in the Peruvian literature it has been correctly identified (Vegas, 1968; Peña, 1971b; Alamo & Valdivieso, 1987, 1997; Paredes et al., 1999). Surprisingly, since its shells are similar to others in the confusing *N. aspera* group, it has never been synonymized with other members. Instead, it has been suggested as a possible southern subspecies of the *N. modesta* group (Keen, 1971; Vermeij, 1973; Finet, 1985), probably on account of the spotted shell pattern. Others have synonymized it with *N. araucana* from Peru and Chile (Tryon, 1887; Dall, 1909; Hertlein & Strong, 1955b; doubtfully by Reid, 1989a, and Finet, 1994).

**Diagnosis:** Shell moderately large, whorls slightly rounded, spire profile straight; 7–11 primary spiral grooves; sculpture of numerous fine grooves (11–23 above periphery of last whorl), but often obsolete on last whorl; white to cream, with pattern of smudged brown dots and broad spiral grey to black-brown band just above periphery (paler on last whorl). Penis with wrinkled filament taper-



ing to point; large mamilliform gland and small glandular disc borne on projection of base.

**Material examined:** 51 lots (including 18 penes, 6 sperm samples, 4 pallial oviducts, 1 spawn sample, 5 radulae).

**Shell (Figures 12R-AA):** Mature shell height 3.2–15.9 mm (to 19.7 mm, Peña, 1971b). Shape high-turbinate to elongate ( $H/B = 1.35\text{--}1.87$ ,  $SH = 1.52\text{--}2.11$ ); spire whorls slightly rounded, suture distinct; spire profile straight; periphery angled, becoming rounded on last whorl. Columella almost straight, slightly hollowed and pinched at base; eroded parietal area. Sculpture of 7–11 primary spiral grooves on spire whorls; primary ribs subequal, or slightly narrower posteriorly (especially if division begins on penultimate whorl); most or all ribs become divided on last whorl, giving 11–23 grooves above periphery, irregularly spaced; grooves remain as incised lines only (rarely enlarging to half width of ribs near periphery) and often become obsolete on shoulder or throughout last whorl; peripheral rib twice as wide as others, but not raised; spiral microstriae absent. Protoconch 0.34 mm diameter, 2.7 whorls. Color very variable; ground color white to cream; usually with broad black-brown spiral band on lower half of spire whorls, becoming blue-grey and diffuse on last whorl; sometimes brown band continues to last whorl, extending to just above or just below periphery, but no separate spiral band present on base; sometimes spiral band is blue-grey and diffuse throughout teleoconch; most shells have pattern of fine smudged brown dots arranged in oblique axial series (occasionally fused to give fine zigzag lines) across whole surface, usually pale on spire and darkening on last whorl; dots sometimes pale or absent; occasionally shells are dark black-brown throughout, with only paler zone at suture and on base, where dots and flames of orange-brown are visible; aperture brown, with 2 pale spiral bands at base and shoulder; columella brown.

**Animal:** Head grey to black, no unpigmented stripe across snout, tentacle pale around eye and usually at inside of base, with two broad longitudinal black stripes meeting at black tip; sides of foot grey or black speckled. Opercular ratio 0.34–0.39. Penis (Figures 14L–P): filament moderately long, tapering toward pointed tip, with annular wrinkles for most of length, opaque and glandular at base, 0.6–0.8 total length; sperm groove open to tip; glandular disc small, smaller than large mamilliform gland, borne on projection of base; penis unpigmented or only slightly pigmented at base. Euspermatozoa 57–61  $\mu\text{m}$ ; paraspermatozoa (Figure 15R) oval, with single stout rod-pieces with rounded ends, 9–23  $\mu\text{m}$ , projecting from cell, cytoplasm packed with large spherical granules. Pallial oviduct (Figure 15F) with copulatory bursa opening near posterior end of straight section and extending back to albumen gland. Spawn (Figures 15G, H) an asymmetrically biconvex pelagic capsule 300  $\mu\text{m}$  diameter, with

thin projecting circumferential flange, usually a thickened ring on upper side, containing single ovum 84  $\mu\text{m}$  diameter. Protoconch indicates planktotrophic development.

**Radula (Figure 16E):** Relative radular length 2.9–7.2. Rachidian: length/width 1.33–1.75; major cusp elongate, pointed or rounded at tip. Lateral and inner marginal: major cusps elongate, rounded at tip. Outer marginal: 5–8 cusps.

**Habitat:** Abundant in uppermost eulittoral and lower littoral fringe, on exposed and semi-sheltered coasts; rare on sheltered muddy shores; recorded on cliffs and outcrops of sandstone, mudstone, concrete, and volcanic conglomerate. Juveniles appear to settle among barnacles. In Ecuador microsympatric with *N. apicina* on exposed coasts, but zoned mainly below *N. tenuistriata*. Recorded also on mangroves and driftwood, on sandy beaches with rocks, in northern Peru (Peña, 1971a, b), but specimens from wood are smaller than from rocks, and this is evidently an atypical habitat.

**Range (Figure 17):** Costa Rica, southern Colombia to northern Peru. Range limits: Tarcoles, 20 km SW of San Mateo, Costa Rica (BMNH 20001251, 2 specimens); Playa de Manuel Antonio, Puerto Quepos, Costa Rica (BMNH 20001252, 30 specimens); Isla Gorgona and Isla Gorgonilla, Colombia (USNM 819737, 1 specimen); Atacames, Esmeraldas, Ecuador (BMNH 20001253); Anconcito, Peninsula Santa Elena, Guayas, Ecuador (BMNH 20001254); Isla Muerta, Golfo de Guayaquil, Ecuador (USNM 819730); Bahía de Sechura, Piura, Peru (BMNH 20001255, 6 specimens). There are in addition three literature records of localities farther south in Peru: Pimentel, Lambayeque (Peña, 1970; Alamo & Valdivieso, 1987, 1997), Pacasmayo, La Libertad (Vegas, 1968; Alamo & Valdivieso, 1987, 1997) and Islas Guañape, La Libertad (Alamo & Valdivieso, 1987, 1997); these may be reliable, but should be treated with caution owing to possible confusion with *N. araucana*. These records to the south of the normal limit of the TEP (3–6°S, see Discussion) might be connected with the expansion of the tropical zone during El Niño events (see Paredes et al., 1998). This species is abundant only in Ecuador and far northern Peru. Farther north there are isolated records only from Isla Gorgona and Costa Rica; nevertheless, at Puerto Quepos it was moderately common, suggesting a possibly self-sustaining population (personal observation). It has not been found in the Gulf of Panama, despite extensive collecting. The species was listed as present in the Galápagos Islands by Finet (1985, 1991), on the basis of two lots in USNM (USNM 819206, 703292, both from Darwin Station, Santa Cruz, total 9 specimens). These are subsamples of a larger lot (17 specimens, G. J. Vermeij Collection), referred to in an ecological paper by Vermeij (1973). All three lots have been examined and the identification confirmed. Nevertheless, this record from the

Galápagos is considered unreliable (as also concluded by Finet, 1994), since considerable collecting effort at this locality and elsewhere has not found the species in the islands. Vermeij also collected on the mainland of Ecuador at about the same time, and confusion of labels may have occurred.

**Remarks:** Of the other species in the *N. aspera* group, *N. paytensis* most closely resembles *N. apicina* and *N. penicillata* in details of shell sculpture and coloration, penial shape, and paraspermatozoa.

The variation in shell color in *N. paytensis* is more extreme than in other members of the *N. aspera* group. Sometimes both dark brown and grey-white shells can be found together on the shore, and brown juveniles may become pale as adults. Notably, at Punta Carnero on the Peninsula Santa Elena in southern Ecuador all shells were dark brown, or almost black, and of small size (less than 8.5 mm; Figure 12R), whereas from nearby Anconcito, shells of similar size were pale. Furthermore, abrupt color change can occur during the course of growth (Figures 12S, T), so that an ecophenotypic component to the variation seems likely. The shells of *N. paytensis* from Costa Rica are white with a slight grey zone on the spire whorls and only faint grey dots toward the end of the last whorl (Figure 12V), quite distinct from other species found there. Anatomically, the animals from Costa Rica are identical to those from Ecuador.

Although so variable in shell color, the species is nevertheless most readily recognized by the pattern of small brown dots on the last whorl, combined with a broad brown or blue-grey band on the spire, which is seen in most shells. Dots are also seen in sympatric *N. conspersa*, but in that species the ground color of the shell is entirely white, and grooves are much wider and fewer in number, so that no confusion should arise. Of the other sympatric species, confusion is likely with *N. apicina*, although this is less common in Ecuador, which is separated by its pattern of oblique axial stripes, breaking up into fine tessellation over the mid-part of the last whorl, and lack of secondary grooves; shells can, however, be very similar. Identification of males of *N. apicina* can be confirmed by the longer rod-pieces of the paraspermatozoa and the form of the penis, which has a more projecting glandular disc and rounded tip to the filament. The usual black (or grey) and white color of *N. tenuistriata* is generally distinctive, but pale examples might be confused with *N. paytensis*; the shell of *N. tenuistriata* is usually larger and broader than that of *N. paytensis*, with similarly narrow ribs but stronger grooves (which never become obsolete) on the last whorl, and the penial glandular disc is larger. Small shells and juveniles (less than 6 mm) with dark coloration (e.g., Figure 12R) can easily be confused with brown adult *N. santelenae*, with which they can be microsympatric among barnacles. Separation is achieved by the rounded periphery and more patulous shape of *N.*

*santelenae*, which has fine microstriae and may develop raised ribs, contrasting with the smooth, glossy surface and incised spiral lines of *N. paytensis*.

There has been a history of confusion of *N. paytensis* with the southern *N. araucana*; these two are restricted to the Panamic and Peruvian Provinces respectively, but in the transitional zone they may occur sympatrically (e.g., records of both from Paíta and Pimentel by Peña, 1970). The shell of the latter species is smaller with a more rounded periphery and slightly produced anterior lip; the color pattern is also variable but never shows axial series of dots; most usefully, the interior of the aperture shows a single pale basal band (two in *N. paytensis*) and in addition penial shape is diagnostic.

### Remaining *Nodilittorina* Species

The remaining species in the eastern Pacific Ocean (*N. araucana*, *N. peruviana*, *N. galapagensis*, *N. fernandezensis*) form a heterogeneous group. The two southern species, *N. araucana* and *N. peruviana*, share some similarities in their shell shape, sculpture, and color pattern, but anatomical characters differ significantly. The Galápagos endemic, *N. galapagensis*, is the only nodulose species in the region and has a unique penial shape. The last species, *N. fernandezensis*, is endemic to the Islas Juan Fernández and Desventuradas off Chile, and is clearly related to a group of species in the southern Pacific rather than to any others in the eastern Pacific.

### *Nodilittorina araucana* (d'Orbigny, 1840)

(Figures 16G, H, 18A–J, 19A–C, F, H, J, K, 20)

- Littorina araucana* d'Orbigny, 1840:393–394; Atlas (1840) pl. 53, figs. 8–10 (Valparaíso, Chili, also entire coast as far as Arica, Pérou [Valparaíso, Chile, to Arica, Chile]; here restricted to Valparaíso, the locality of the types; lectotype (here designated, 7.0 × 4.7 mm) BMNH 1854.12.4.365/1, seen, Figure 18I; 12 paralectotypes BMNH 1854.12.4.365/2, seen, 1 is probably not this species). Hupé, 1854:138. Reeve, 1857:sp. 88, pl. 16, fig. 88. Dall, 1909:231, 285 (in part, includes *N. paytensis*). Keen, 1971:365.
- Littorina araucana*—Philippi, 1847:2:197, *Littorina* pl. 4, fig. 5. Küster, 1856:17, pl. 2, figs. 21, 22 (1856). Weinkauff, 1878:30 (as *auracana*). Weinkauff, 1883:219. Strebel, 1907:155–156.
- Littorina (Melaraphe) araucana*—H. & A. Adams, 1854:314.
- Littorina (Austrolittorina) araucana*—Rosewater, 1970:423. Dell, 1971:205. Marinovich, 1973:25, figs. 48, 49. Alamo & Valdivieso, 1987:25. Alamo & Valdivieso, 1997:17.
- Nodilittorina (Nodilittorina) araucana*—Reid, 1989a:99 (*N. paytensis* a doubtful synonym). Skoglund, 1992:15 (*N. paytensis* a doubtful synonym).
- Nodilittorina araucana*—Finet, 1994:18, 127 (*N. paytensis* doubtfully included). Reid & Osorio, 2000:123, fig. 7C.
- Littorina thersites* Reeve, 1857:sp. 78, pl. 15, figs. 78a, b (Valparaíso [Chile]; 4 syntypes BMNH 1968317, Figure

18C, seen). Weinkauff, 1882:69, pl. 9, figs. 5, 8. Weinkauff, 1883:219. Dall, 1909:231.

*Littorina (Melaraphe) thersites*—Tryon, 1887:252, pl. 45, fig. 18 (doubtfully placed in synonymy of *L. (M.) neritoides*).

*Littorina (Melaraphe) paytensis*—Tryon, 1887:250, pl. 45, figs. 95, 96 (in part, includes *N. paytensis*).

**Taxonomic history:** The shell of this species is highly variable; d'Orbigny's (1840) species was based on elongate, faintly striated, brown shells, and that of Reeve (1857) on low-spired, grooved, blue-grey shells, although both collections were from Valparaíso. One of the paralectotypes of *L. araucana* is probably not this species; it bears fine oblique axial stripes over the whole whorl width, but is too eroded for certain identification. The name *thersites* has seldom been used; Weinkauff (1882, 1883) and Dall (1909) both accepted it as a distinct species, whereas Tryon (1887) doubtfully placed it in the synonymy of the European *Melarhaphe neritoides*. In Peru and Chile *N. araucana* is well known. The only taxonomic confusion has arisen from its superficial similarity to some shells of *N. paytensis*, a species that was doubtfully included in the synonymy of *N. araucana* by Reid (1989a, followed by Finet, 1994); Tryon (1887) disregarded priority and used the name *paytensis* for this species.

**Diagnosis:** Shell small, whorls rounded, spire profile straight to slightly convex, periphery rounded; slightly produced anterior lip; six to 10 primary spiral grooves; sculpture of numerous fine grooves (up to 29 in total on last whorl), but often obsolete on last whorl; white to dark brown, pale basal band; single pale basal band within brown aperture. Penial filament broad, with subterminal opening of sperm groove; mamilliform gland and small glandular disc borne on projection of base.

**Material examined:** 52 lots (including 16 penes, 6 sperm samples, 6 pallial oviducts, 2 spawn samples, 4 radulae).

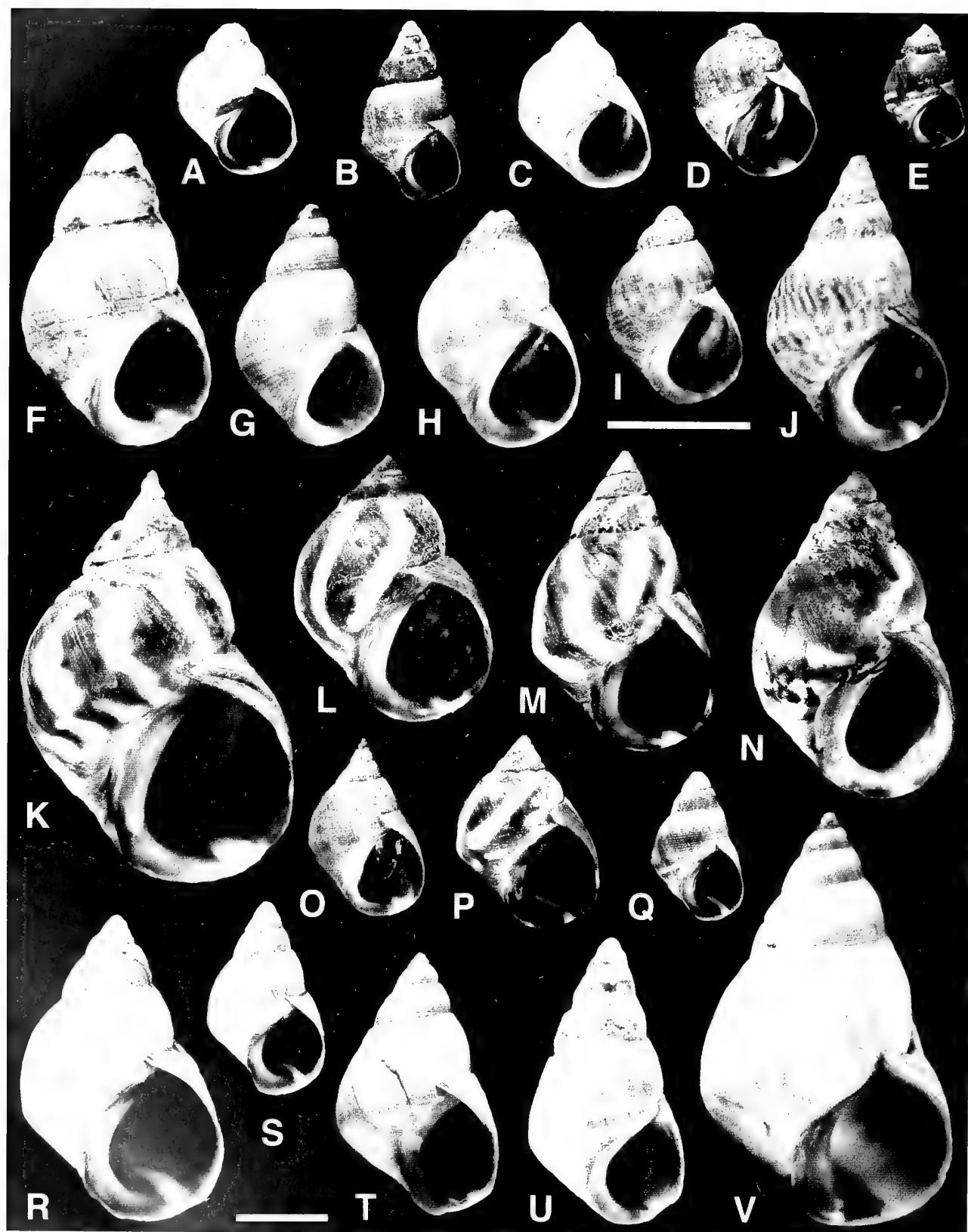
**Shell (Figures 18A–J):** Mature shell height 1.5–13.8 mm. Shape globular to elongate ( $H/B = 1.13$ – $1.89$ ,  $SH = 1.39$ – $2.38$ ); spire whorls rounded, suture distinct; spire profile straight to slightly convex; periphery usually rounded, sometimes angled. Columella concave to straight, slightly hollowed and pinched at base, anterior lip often slightly produced; small eroded parietal area. Sculpture of 6–10 primary spiral grooves on spire whorls, slightly more closely spaced posteriorly; secondary sculpture may start early, on last whorl up to 29 grooves in total (including base), irregularly spaced, grooves usually remain as incised lines, exceptionally equal to width of intervening ribs, peripheral rib may be slightly raised; more frequently sculpture becomes obsolete above periphery on last whorl, or over entire whorl; spiral microstriae absent; spire frequently eroded. Periostracum relatively thick, slightly overhanging apertural edge. Protoconch 0.37 mm diameter, about 3 whorls. Color white

to blue-grey to black-brown; dark brown shells may be paler near suture and on base, with white basal band; definite color pattern usually limited to white basal band, occasionally broken up into spots, and rarely with coarse white marbling over entire base; exceptionally 1–2 white spiral bands above periphery at end of last whorl and 2 below (including normal basal band); axial pattern rarely present, never showing distinct opisthocline oblique stripes, but limited to differentially colored growth lines, or slightly wavy lines along prosocline growth increments; aperture brown, with single pale spiral band at base; columella brown.

**Animal:** Head and tentacles black, pale only around eye; sides of foot dark grey to black. Opercular ratio 0.38–0.50. Penis (Figures 19A–C): filament broad, glandular at base, sometimes reddish at tip, 0.6–0.7 total penial length; sperm groove appears to end subterminally at about 0.7 filament length (although in fact it continues as a shallow trace to the tip), so that filament abruptly narrows behind tapered tip; glandular disc small, smaller than moderate mamilliform gland, borne on projection of base; penis with black pigment at base. Euspermatozoa 64–71  $\mu\text{m}$  (80–100  $\mu\text{m}$ , Jordan & Ramorino, 1975); paraspermatozoa (Figures 19J, K) oval to fusiform, 16–40  $\mu\text{m}$  (35  $\mu\text{m}$ , Jordan & Ramorino, 1975), packed with spherical granules that become smaller toward one (often pointed) end; no rod-pieces visible. Pallial oviduct (Figure 19H) with slight flexure in path of egg groove through opaque capsule gland, copulatory bursa sometimes very large, opening near posterior end of straight section, extending back almost to albumen gland. Spawn (Figure 19F) an asymmetrically biconvex pelagic capsule, 210–256  $\mu\text{m}$  diameter, with broad vertical peripheral rim, single ring on domed upper surface, containing single ovum 68–84  $\mu\text{m}$  diameter (Jordan & Ramorino, 1975). Development planktotrophic (Jordan & Ramorino, 1975).

**Radula (Figures 16G, H):** Relative radular length 1.5–3.5. Rachidian: length/width 1.23–1.45; major cusp moderately elongate, rounded at tip. Lateral and inner marginal: major cusps elongate, rounded or blunt at tip. Outer marginal: 7–8 cusps.

**Habitat:** Throughout most of its range, this species is abundant and characteristic of the bare littoral fringe and uppermost barnacle zone, on both exposed and sheltered rocky coasts (Alveal, 1970, 1971; Romo & Alveal, 1977; Santelices et al., 1977; Ruiz & Giampaoli, 1981). However, at the southern extreme of its range, in the Chilean Archipelago, it has been reported to occur commonly both in the littoral fringe and throughout the eulittoral zone (Dell, 1971; Alveal & Romo, 1977; Brattström, 1990; Reid & Osorio, 2000), among barnacles, tufts of red algae *Hildenbrandia* and *Iridaea*, filamentous green algae, beds of *Mytilus* and *Perumytilus*, and in shallow pools of the upper eulittoral. Most of these southern ob-



servations have been made on sheltered shores, whereas on exposed shores in the region the zonation is apparently more typical, among the uppermost barnacles and *Hildenbrandia* (Reid & Osorio, 2000). The species is often sympatric with *N. peruviana*, with which it may overlap in the upper barnacle zone, but *N. araucana* extends to higher levels in the littoral fringe (Guiler, 1959b; Alveal, 1970, 1971; Santelices et al., 1977; Brattström, 1990). Of these two species, *N. araucana* has been said to be the more common in exposed sites (Romo & Alveal, 1977), but Marinovich (1973) reported that it was absent on the most wave-exposed coasts in the vicinity of Iquique.

**Range (Figure 20):** Peru and Chile. Range limits: Paita, Piura, Peru (Alamo & Valdivieso, 1987, 1997; in view of possible confusion with *N. paytensis* this record might be doubted); Salaverry, La Libertad, Peru (USNM 667199); Forelius Peninsula, Aisen, Chile (BMNH 20001261). Dall (1909) gave the range as Isla Chiloé to Nicaragua, and this northern limit has been quoted by others (Carcelles & Williamson, 1951; Dell, 1971; Alamo & Valdivieso, 1987, 1997); it is undoubtedly incorrect and may partly be explained by the fact that Dall (1909) included *N. paytensis* in his concept of *araucana*, although even that species is not recorded farther north than Costa Rica.

**Remarks:** This species covers a range of latitude from at least 8 to 47°S, and therefore extends throughout the Peruvian Province, including the transitional zone with the southern Magellanic Province. Shell form, sculpture, and color are remarkably variable, and indeed Reeve (1857) described the low-spired, striated, blue-grey form as a species distinct from the taller, smoother brown shells originally described by d'Orbigny (1840). Nevertheless, such variation is common among *Nodilittorina* species, as repeatedly shown in those from the eastern Pacific. Furthermore, intergradation between extreme forms is found at most localities, as also noted by Marinovich (1973). It is not known if there are microenvironmental correlates of the shell variation within localities. However, there appears to be a geographical component to color variation. Of the material examined in the present

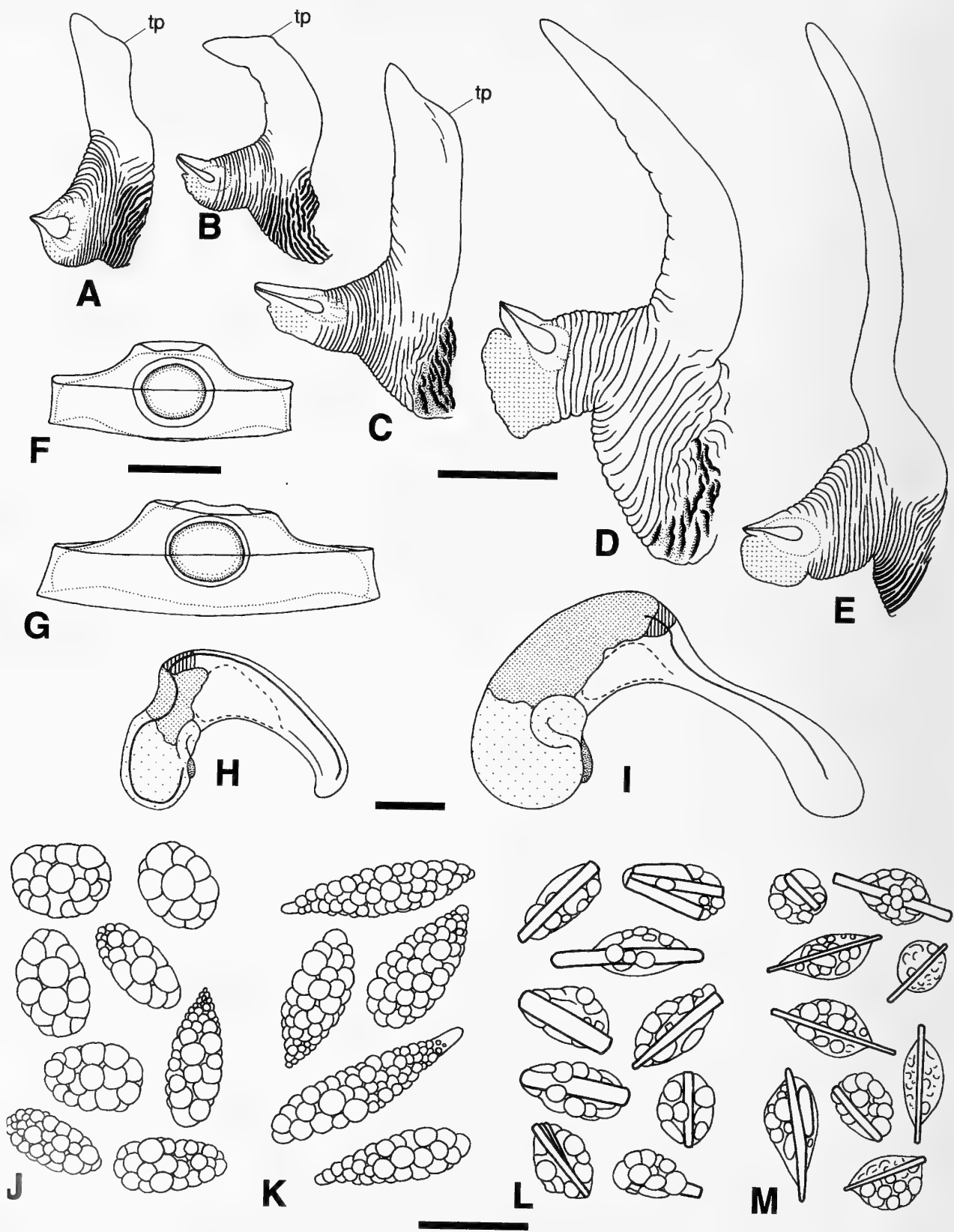
study all 19 collections from south of Valparaíso consisted entirely of brown shells. Only at Valparaíso (33°S) and northward were some blue-grey to white shells found (in 50% of the 26 samples seen), usually mixed with shells of variably brown hue. One possible explanation is that at lower latitudes there is climatic selection in favor of pale shells, which reach lower temperatures in sunlight (see Markel, 1971, for temperature measurements of dark *N. dubiosa* and white *N. conspersa*). Hughes (1979) described a supposedly intraspecific geographical cline from dark brown to blue-grey shells with decreasing latitude along the east coast of South Africa, although it is now known that two distinct species are involved (dark *N. knysnaensis* and pale *N. africana*; unpublished observations). Note that in dry museum collections the brown color of the shell fades to beige.

This species is unlikely to be confused with its frequently sympatric but much larger congener *N. peruviana*, except when the latter is small and lacking its striking zebra pattern (see Remarks on *N. peruviana*). Similarity to *N. paytensis*, with which it is occasionally sympatric in northern Peru, has been discussed in the Remarks on that species.

The relationships of *N. araucana* are unclear. Some features, such as the tendency toward a smooth shell, rather irregular pattern (even if often restricted to the base), shape of the aperture, and form of the spawn resemble the sympatric *N. peruviana*. However, in other respects *N. araucana* does not appear to be closely related to other *Nodilittorina* species from Central and South America. The peculiar subterminal opening of the sperm groove and slight flexure in the capsule gland are unusual, while the form of the paraspermatozoa is, so far as is known, unique in the genus. A similar penial shape is seen in *N. riisei* (Mørch) from the Caribbean and also in *N. unifasciata* from Australia (but the slight flexure of the egg groove in the capsule gland of *N. araucana* does not closely resemble the large loop in this position in members of *Austrolittorina*, see Remarks on *N. fernandezensis*).

←

Figure 18. Shells of *Nodilittorina araucana* (A–J), *N. peruviana* (K–P), and *N. fernandezensis* (Q–V). A. Tubul, Bío-Bío, Chile (BMNH 20001262). B. Isla Acuaco, Aisen, Chile (BMNH 19990379). C. Syntype of *Littorina thersites* Reeve, 1857; Valparaíso, Chile (BMNH 1968317). D. Mollendo, Arequipa, Peru (BMNH 1938.7.11.56). E. Las Cruces, Valparaíso, Chile (BMNH 20001263). F, G. Coquimbo, Chile (BMNH 1886.6.9.259, 260). H. Los Vilos, Coquimbo, Chile (BMNH 20001264). I. Lectotype of *Littorina araucana* d'Orbigny, 1840; Valparaíso, Chile (BMNH 1854.12.4.365/1). J. Taltal, Antofagasta, Chile (USNM 713998). K. Valparaíso, Chile (BMNH 20001267). L. Locality unknown (BMNH 20001268). M. Locality unknown (BMNH 20001269). N. Lectotype of *Phasianella peruviana* Lamarck, 1822; Callao, Lima, Peru (MHNG 1096/86-1). O. Salaverry, La Libertad, Peru (USNM 667199). P. Las Cruces, Valparaíso, Chile (BMNH 20001270). Q. Isla Alejandro Selkirk, Archipiélago de Juan Fernández (BMNH 20001278). R. S. Punta San Carlos, Isla Robinson Crusoe (Más a Tierra), Archipiélago de Juan Fernández (BMNH 20001279). T. Holotype of *Littorina unifasciata fernandezensis* Rosewater, 1970; Bahía Cumberland, Isla Más a Tierra, Archipiélago de Juan Fernández (USNM 368900). U. Locality unknown (BMNH 1914.5.8.6). V. Isla San Felix, Islas Desventuradas (BMNH 20001280). Scale bars A–J = 5 mm; K–V = 5 mm.





*Nodilittorina peruviana* (Lamarck, 1822)

(Figures 16F, 18K–P, 19D, E, G, I, L, M, 20)

*Phasianella peruviana* Lamarck, 1822:53 (les côtes du Pérou, près de Callao [Callao, Peru]; lectotype (here designated) MHNG 1096/86-1, 16.7 × 11 mm, Figure 18N; 1 paralectotype MHNG 1096/86-2; 1 lost paralectotype figured by Delessert, 1841:pl. 37, fig. 9a, b). Delessert, 1841:pl. 37, fig. 9a, b. Deshayes & Milne Edwards, 1843:243.

*Littorina peruviana*—Gray, 1839:138, pl. 36, fig. 8 (includes *Littorina striata* King & Broderip, 1832 in synonymy). d'Orbigny, 1840:393, pl. 53, figs. 5–7. Hupé, 1854:137–138. Stearns, 1893b:444. Dall, 1909:172–173, 231, 285, pl. 23, fig. 7. Vegas, 1968:7–8. Keen, 1971:366, fig. 185. Finet, 1985:9, 13.

*Littorina (Melaraphe) peruviana*—H. & A. Adams, 1854:314. Tryon, 1887:249, pl. 44, fig. 78.

*Littorina peruviana*—Weinkauff, 1883:221.

*Littorina (Austrolittorina) peruviana*—Rosewater, 1970:423. Marincovich, 1973:26, fig. 50. Basly, 1983:18, pl. 4, fig. 34. Alamo & Valdivieso, 1987:25, fig. 36. Alamo & Valdivieso, 1997:17, fig. 36.

*Nodilittorina peruviana*—Bandel & Kadolsky, 1982:14. Finet, 1994:18.

*Nodilittorina (Echinolittorina) peruviana*—Reid, 1989a:99. Skoglund, 1992:15. Kaiser, 1997:27.

*Turbo zebra* Wood, 1828:20, pl. 6, *Turbo* fig. 33 (S. America; here restricted to Arica, Chile, the locality of the syntypes; 18 syntypes, BMNH 1968367, seen).

*Littorina zebra*—Philippi, 1847:2:165, *Littorina* pl. 3, fig. 16. Küster, 1856:18–19, pl. 2, figs. 25–27 (1853). Weinkauff, 1878:31.

*Littorina zebra*—Reeve, 1857:sp. 61, pl. 12, fig. 61a, b. Troschel, 1858:134, pl. 11, fig. 2 (radula).

*Littorina zebra* var. *nana* Nevill, 1885:140 (Valparaíso [Chile]; *nomen nudum*).

**Taxonomic history:** This well known species is sufficiently distinctive that it has not been confused with any other. In the early literature the junior synonym *zebra* was commonly employed by German and English authors (an exception was Gray, 1839), but following the revisions of Weinkauff (1883) and Tryon (1887) the earlier *peruviana* prevailed.

**Diagnosis:** Shell large, whorls rounded, spire profile concave near apex, periphery rounded; slightly produced anterior lip; spiral sculpture usually absent; white with few broad irregular waved axial black stripes; single pale basal

band within brown aperture. Penial filament long; mamilliform gland and large glandular disc borne on projection of base.

**Material examined:** 63 lots (including 11 penes, 5 sperm samples, 4 pallial oviducts, 4 radulae).

**Shell (Figures 18K–P):** Mature shell height 6.0 mm (Jordan & Ramorino, 1975)–23.8 mm. Shape high turbate (H/B = 1.31–1.88, SH = 1.46–1.93); spire whorls rounded, suture distinct; spire profile concave near apex, but often eroded; periphery rounded, or only slightly angled in some juveniles. Columella long, concave to straight, slightly hollowed and pinched at base, anterior lip often slightly produced; no eroded parietal area. Spiral sculpture usually absent, even on early whorls; sometimes 1–4 (rarely 6–8) faint incised lines above periphery; occasionally a slightly enlarged rib forms an angle at periphery; spiral microstriae absent. Periostracum relatively thick, sometimes slightly overhanging apertural edge. Protoconch not seen. Color white with dark brown or black broad, waved or irregular axial stripes; sometimes entirely black or with only an interrupted white basal band; aperture purplish black, with single pale spiral band at base; columella brown to black.

**Animal:** Head and tentacles black, pale around eye and sometimes at inside of tentacle base; sides of foot dark grey to black. Opercular ratio 0.36–0.43. Penis (Figures 19D, E): filament long when fully relaxed, 0.7–0.8 total length, sperm groove open to tip; glandular disc large, often projecting, borne with mamilliform gland on projection of base; penis unpigmented or slightly pigmented at base. Euspermatozoa 57–64 µm (65–80 µm, Jordan & Ramorino, 1975); paraspermatozoa (Figures 19L, M) oval, 11–18 µm (25 µm, Jordan & Ramorino, 1975), filled with large round granules, often an apparent nucleus visible, rod-pieces variable within individuals, single or multiple, short and rectangular or long slender and projecting, up to 28 µm long. Pallial oviduct (Figure 19I) with copulatory bursa opening near posterior end of straight section and extending back to albumen gland. Spawn (Figure 19G) an asymmetrically biconvex pelagic capsule, 336–421 µm diameter, with broad and slightly oblique peripheral rim, 1–2 rings on domed upper surface, containing single ovum 84–89 µm diameter (Jordan

Figure 19. Penes (A–E), egg capsules (F, G), pallial oviducts (H, I), and paraspermatozoa (J–M) of *Nodilittorina araucana* (A–C, F, H, J, K) and *N. peruviana* (D, E, G, I, L, M). A. Isla Acuaco, Aisen, Chile (BMNH 19990379; shell H = 7.2 mm). B, C, H, J. Las Cruces, Valparaíso, Chile (BMNH 20001263; shell H = 7.0 mm, 6.7 mm, 8.2 mm). D. Playa La Lisera, Arica, Tarapacá, Chile (BMNH 20001271; shell H = 11.9 mm). E, I. Caleta Catarindo, Arequipa, Peru (BMNH 20001272; shell H = 11.9 mm, 13.0 mm). F. Montemar, Valparaíso, Chile (after Jordan & Ramorino, 1975). G. Montemar, Valparaíso, Chile (after Jordan & Ramorino, 1975). K. Concepción, Bío-Bío, Chile (BMNH 20001265). L, M. Las Cruces, Valparaíso, Chile (BMNH 20001270). Abbreviation: tp, termination of deep part of penial sperm groove (continues as a shallow trace to tip). Shading conventions as in Figures 3, 4, 13. Scale bars: A–E = 1 mm; F, G = 0.1 mm; H, I = 1 mm; J–M = 20 µm.



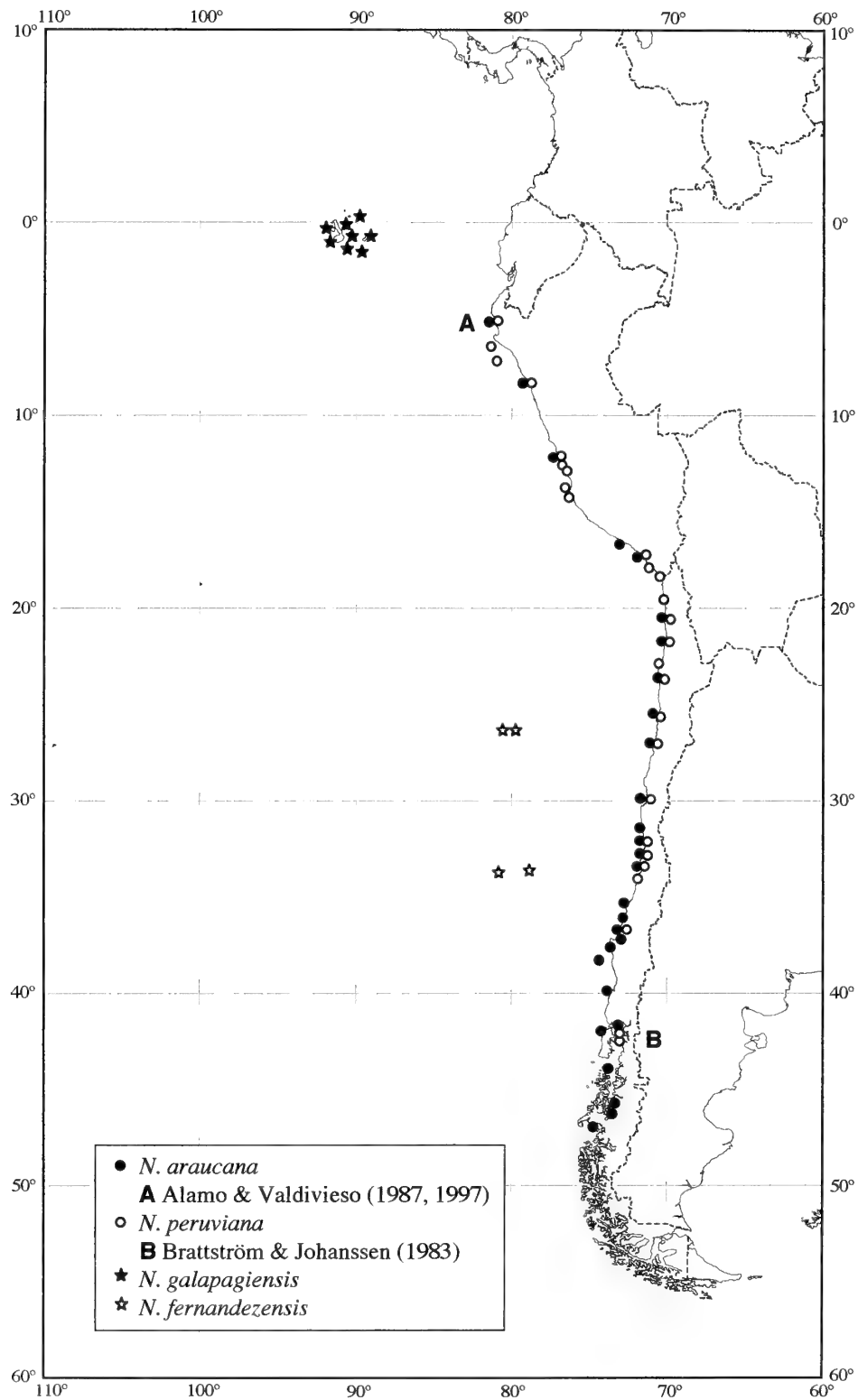


Figure 20. Geographical distribution of *Nodilittorina araucana*, *N. peruviana*, *N. galapagensis*, and *N. fernandezensis* (records based on material examined and quoted literature sources).

& Ramorino, 1975). Development planktotrophic (Jordan & Ramorino, 1975).

**Radula (Figure 16F):** Relative radular length 2.8–3.4. Rachidian: length/width 1.34–1.41; major cusp elongate, rounded at tip. Lateral and inner marginal: major cusps elongate, rounded at tip. Outer marginal: 7–10 cusps.

**Habitat:** Abundant on rocky coasts, both exposed and sheltered, but favoring strong wave-exposure; typically in upper part of barnacle zone, extending into littoral fringe in areas with heavy spray; also among *Perumytilus*; often sympatric with *N. araucana*, but zoned slightly below that species (Guiler, 1959a, b; Vegas, 1963; Alveal, 1970, 1971; Marincovich, 1973; Paredes, 1974; Romo & Alveal, 1977; Santelices et al., 1977; Brattström, 1990). The algal diet has been studied by Santelices & Ugarte (1987).

**Range (Figure 20):** Peru and Chile. Range limits: Paita, Piura, Peru (USNM 6029, 2 specimens; Stearns, 1891; Vegas, 1963; Peña, 1970; Alamo & Valdivieso, 1987, 1997); Isla Lobos de Tierra, Piura, Peru (USNM 538007, 4 specimens; Alamo & Valdivieso, 1987, 1997); Isla Lobos de Afuera, Lambayeque, Peru (USNM 753012, 4 specimens); Navidad, Santiago, Chile (ZMA); Río Bío-Bío, Concepción, Chile (BMNH 20001266, 1 specimen); Isla Chiloé, Chile (USNM 348499, 2 specimens); Golfo de Ancud, 41°49'S, Chile (Brattström & Johanssen, 1983). Records include islands such as Isla Lobos de Afuera (60 km from the mainland). This species appears to be scarce south of the Santiago Region, from which only three museum specimens have been seen, whereas *N. araucana* is relatively frequently represented from the same area. Brattström (1990) observed that it was uncommon in Seno Reloncaví (southern Chile). The northernmost record from Paita is based on an old sample in USNM, but the locality is confirmed by the authors quoted above. This locality is close to the northern limit of the influence of the cold Peru Current (see Discussion). Two old collections from the Galápagos Islands (USNM 60661; USNM 132798) are apparently the basis for the records by Stearns (1893b) and Dall (1909) that have been quoted by subsequent authors (Keen, 1971; Finet, 1985; Alamo & Valdivieso, 1987, 1997). Finet (1994) doubted the provenance of the specimens, yet curiously accepted the literature records based on them. He therefore counted the species as one of only three “purely Peruvian” members of the Galápagos fauna (Finet, 1991, 1994). Occurrence in the Galápagos has not been verified by any recent records, despite considerable collecting effort, and is here regarded as unlikely. Dall (1909) also mentions the species from Nicaragua and Panama, where it certainly does not occur.

**Remarks:** The large size and strikingly colored, smooth shell of this species are so distinctive that confusion of typical specimens with any other species is not likely. Juveniles can be confused with the smaller sympatric *N.*

*araucana* if they have entirely black shells; such shells are recognized by the delicate apertural edge (indicating that they are juvenile) and their distinctively concave spire profile.

The possible relationships of this species are unclear. While the shell shows some similarity to that of *N. araucana* in its frequently smooth surface, apertural shape, and irregular pattern, and in egg capsule morphology, the anatomical differences in paraspermatozoa and penial shape are likely to be more significant. *Nodilittorina peruviana* is similar to the members of the *N. aspera* group in the form of the penis, oviduct, and paraspermatozoa, but these are all of types common throughout the genus and so do not necessarily indicate close relationship.

### *Nodilittorina galapagensis* (Stearns, 1892)

(Figures 7L–Q, 20, 21A–F, K, M, N, 22A, B)

*Hamus lemniscatus*—Wimmer, 1880:32 (not *Littorina lemniscata* Philippi, 1846 = *N. miliaris* (Quoy & Gaimard, 1833)).

*Tectarius lemniscatus*—Stearns, 1893b:397, 444 (not Philippi, 1846).

*Hamus trochoides*—Wimmer, 1880:32–33 (not *Littorina trochoides* Gray, 1839 = *N. trochoides*).

*Tectarius trochoides*—Stearns, 1893b:397, 444 (not Gray, 1839).

*Littorina* (*Tectarius*) *galapagensis* Stearns, 1892:87–88 (James Island [Isla Santiago, Galápagos]; holotype USNM 102509, Stearns, 1892:pl. 51, fig. 7, Figure 7L herein, seen). Stearns, 1893b:396–397, pl. 51, fig. 7.

*Tectarius galapagensis*—Pilsbry & Vanatta, 1902:553. Dall, 1909:232. Hertlein & Strong, 1939:371. Hertlein & Strong, 1955a:137.

*Nodilittorina* (*Nodilittorina*) *galapagensis*—Rosewater, 1970:424. Reid, 1989a:99. Skoglund, 1992:15. Kaiser, 1993:106. Kaiser, 1997:27.

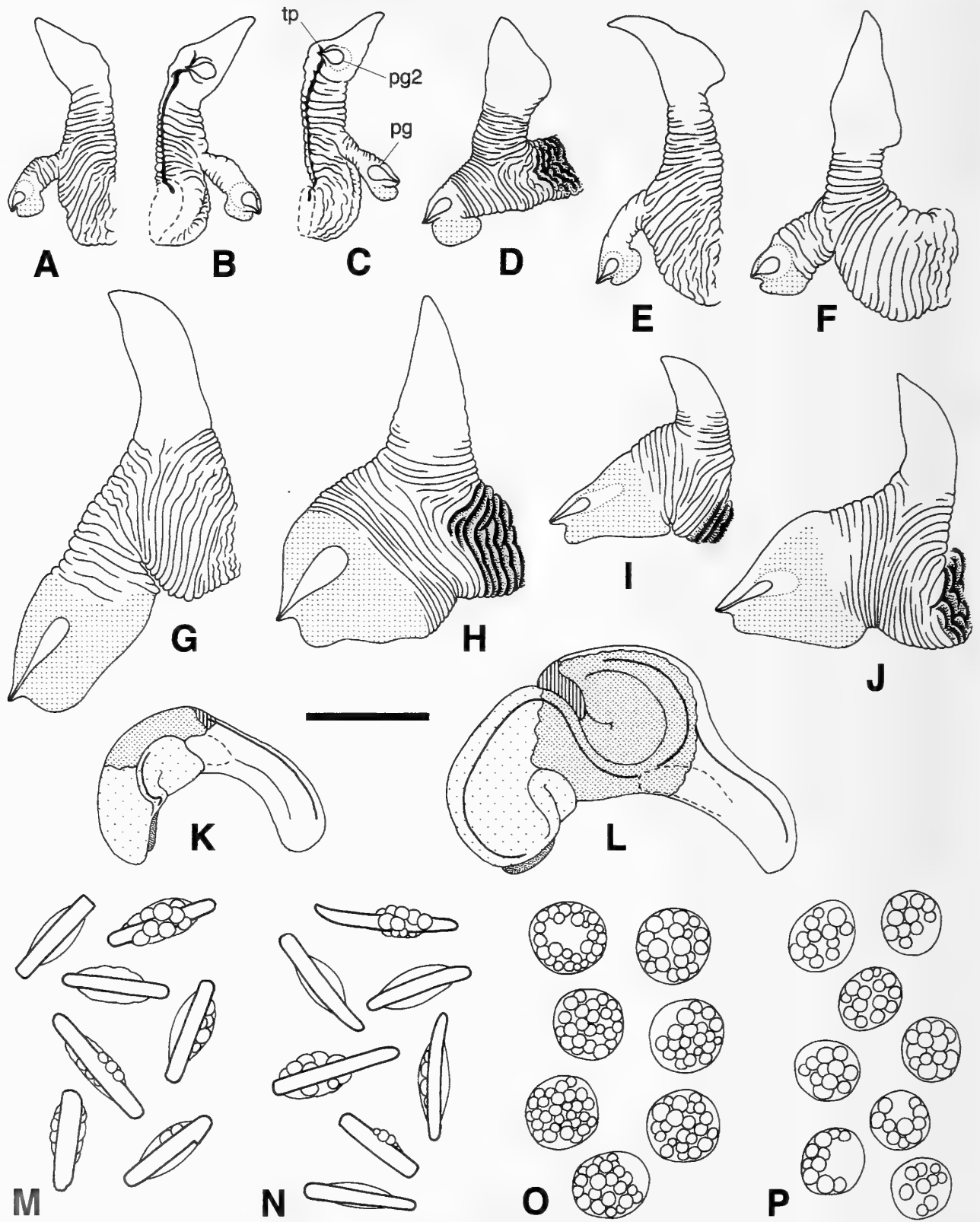
*Nodilittorina galapagensis*—Keen, 1971:367, fig. 190. Hertz, 1977:29, fig. Taviani, 1979:14, figs. 10A, B, 11. Finet, 1985:13. Finet, 1994:18.

*Tectarius atyphus* Stearns, 1891:326 (*nomen nudum*).

*Littorina* (*Tectarius*) *atyphus* Stearns, 1892:88–89 (Manta, Ecuador; holotype USNM 48396, seen).

*Tectarius atyphus*—Stearns, 1893a:350, pl. 50, fig. 5.

**Taxonomic history:** This nodulose shell was confused with similar forms from the Atlantic and West Pacific by Wimmer (1880; quoted by Stearns, 1893b). It is surprising that Stearns (1892) should have described this species twice, as *galapagensis* and *atyphus*, in the same publication. However, he possessed only single specimens of each, and the latter was a more elongate, less nodulose beachworn shell, said to have originated from Manta on the mainland of Ecuador. Only Stearns (1891, 1892, 1893a) employed the name *atyphus*; subsequent authors (following Pilsbry & Vanatta, 1902, as first revisers) have recognized its synonymy with *N. galapagensis*. Like other members of *Nodilittorina* with nodulose sculpture, this has in the past sometimes been placed in the genus *Tectarius* (= *Hamus*; see Rosewater, 1972; Reid, 1989a).



**Diagnosis:** Shell small; sculpture of nodulose or granulose cords; dark brown to black with white band on base, nodules whitish, especially on base. Penial filament with hooked tip, subterminal opening of sperm groove; small mamilliform gland and small glandular disc borne on narrow projection of base; a second mamilliform gland on medial side of filament, behind tip.

**Material examined:** 33 lots (including 12 penes, 3 sperm samples, 4 pallial oviducts, 4 radulae).

**Shell (Figures 7L–Q):** Mature shell height 3.5–13.1 mm. Shape turbanate to high turbanate ( $H/B = 1.23\text{--}1.79$ ,  $SH = 1.58\text{--}2.19$ ); spire whorls usually rounded, suture distinct, sometimes flattened and with indistinct suture; spire profile straight; periphery rounded or angled, marked by nodulose rib. Columella short, concave, hollowed, and slightly pinched at base, anterior columellar lip slightly flared; small eroded parietal area. Sculpture of 3 spiral rows of nodules (at periphery, shoulder and, smaller, near suture); base with 2–5 nodulose or granulose cords; nodules usually large, but may be reduced in size to give granulose rather than nodulose appearance to shell, occasionally reduced to mere undulations on fine spiral riblets numbering about 20 on last whorl; entire surface covered with fine spiral microstriae grading into riblets. Protoconch 0.26 mm diameter, 2.8 whorls. Color dark brown to black, nodules often paler brown or, on base, whitish; spiral cream band on base and often a pale line on shoulder, sometimes also at suture; aperture dark brown, pale spiral band at base; columella purplish brown.

**Animal:** Head black; tentacle with 2 broad black longitudinal stripes, pale around eye, at tip and at inside of tentacle base; sides of foot black. Opercular ratio 0.44–0.60. Penis (Figures 21A–F): filament wrinkled at base, about 0.5–0.6 total length, dilated distally with hooklike tip, opening of sperm groove subterminal on raised projection; small glandular disc and small mamilliform gland on narrow projection of base; second mamilliform gland on medial surface of distal part of filament, surrounded by glandular subepithelial tissue; penis unpigmented. Euspermatozoa 39–43  $\mu\text{m}$ ; paraspermatozoa (Figures 21M, N) oval with single long straight (or slightly curved) blunt projecting rod-pieces, 16–25  $\mu\text{m}$ , cyto-

plasm filled with large round granules. Pallial oviduct (Figure 21K) with copulatory bursa opening near posterior end of straight section and extending back to albumen gland. Spawn not observed. Protoconch indicates planktotrophic development.

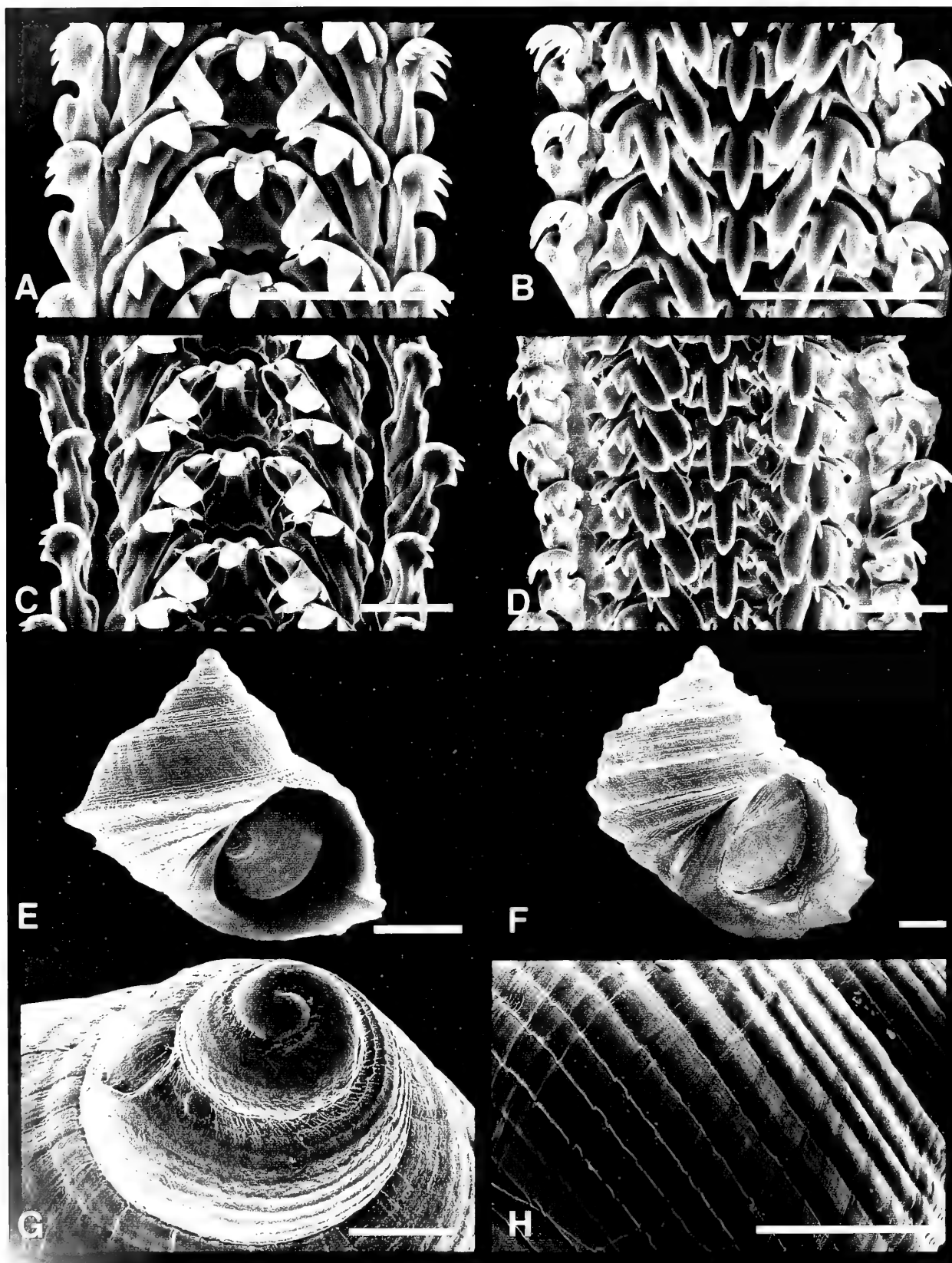
**Radula (Figures 22A, B):** Relative radular length 1.6–5.5. Rachidian: length/width 1.39–1.57; major cusp elongate, pointed or slightly rounded at tip. Lateral and inner marginal: major cusps elongate, rounded or pointed at tip. Outer marginal: 9–10 cusps.

**Habitat:** Abundant on boulders, rocks and cliffs of lava, also on volcanic tuff and on concrete; on bare surfaces in littoral fringe and uppermost eulittoral zone, also in crevices and at edges of saline pools; on exposed and sheltered shores. This is the only abundant littorinid of the littoral fringe in the Galápagos Islands; sympatric *N. conspersa*, *N. atrata*, and *N. porcata* are all found at lower levels. For descriptions of zonation see Cinelli & Colantoni (1974, as *Tectarius galapagensis*); these authors also record occurrence on mangrove trunks.

**Range (Figure 20):** Probably endemic to the Galápagos Islands; a single record from mainland Ecuador (Stearns, 1891, 1892) has not been confirmed. Range limits: Caleta Iguana, Isla Isabela (USNM 796177); NE side Isla Fernandina (LACM AHF 153–34); Punta Egas, Isla Santiago (USNM 807237); Isla Bartolomé (USNM 707612); Isla Genovesa (personal observation); Puerto Ayora, Isla Santa Cruz (USNM 769823; BMNH 20001273); Punta Pitt, Isla San Cristobal (personal observation); Bahía Gardner, Isla Española (CDRS); Punta Cormorant, Isla Santa María (personal observation). Stearns (1891, 1892) recorded a single specimen from Manta on the mainland of Ecuador (as *Tectarius atyphus*; USNM 48396), said to have been collected there by W. H. Jones. Since then, no further specimens are known to have been collected on the mainland. It is possible that some error of labeling occurred, for in the same month (August 1884) the navy surgeon visited both Manta and the Galápagos Islands.

**Remarks:** Specimens with well developed nodules cannot be mistaken for any other littorinid from the region. Small, relatively smooth specimens might be confused with brown shells of the sympatric *N. atrata* and *N. por-*

Figure 21. Penes (A–J), pallial oviducts (K, L), and paraspermatozoa (M–P) of *Nodilittorina galapagensis* (A–F, K, M, N) and *N. fernandezensis* (G–J, L, O, P). A–D, K, M, N. Puerto Ayora, Isla Santa Cruz, Galápagos Islands (BMNH 20001273; shell H = 6.0 mm, 4.6 mm, 6.5 mm, 8.8 mm; A, B, two views). E, F. Punta Estrada, Isla Santa Cruz, Galápagos Islands (E, BMNH 20001274, shell H = 7.4 mm; F, BMNH 20001276, shell H = 6.9 mm). G. Punta San Carlos, Isla Robinson Crusoe (Más a Tierra), Archipiélago de Juan Fernández (BMNH 20001279; shell H = 12.8 mm). H. Isla San Ambrosio, Islas Desventuradas (BMNH 20001281; shell H = 17.0 mm). I, J, L, O, P. Bai del Oeste, Isla Robinson Crusoe (Más a Tierra), Archipiélago de Juan Fernández (BMNH 20001282; shell H = 7.5 mm, 10.7 mm, 13.7 mm). Abbreviations: pg, mamilliform penial gland in normal position; pg2, second mamilliform penial gland; tp, termination of penial sperm groove. Shading conventions as in Figures 3, 4, 13. Scale bars: A–L = 1 mm; M–P = 20  $\mu\text{m}$ .



*cata*, but both of these usually have a lower spire and a large pseudo-umbilicus. Stearns (1892, 1893a, b) described nodulose and granulose forms under different names, and the variability in development of nodules was noted by Taviani (1979).

The nodulose shell is very distinctive and unlike that of any other eastern Pacific species, while it superficially resembles such nodulose Atlantic species as *N. dilatata*, *N. miliaris*, and *N. granosa* (Philippi). However, sculpture is so variable within many *Nodilittorina* species that it appears to be an unreliable guide to relationships. Of more significance is the penis which, with its unusual subterminally opening sperm groove and unique second mamilliform gland, does not closely resemble that of any other species.

*Nodilittorina (Austrolittorina) fernandezensis*  
(Rosewater, 1970)

(Figures 18Q–V, 20, 21G–J, L, O, P, 22C, D)

*Littorina penitaria* "Wood" Nevill, 1885:142 (San Juan Fernandez [Juan Fernandez Islands]; *nomen nudum*).

*Littorina mauritiana*—Odhner, 1922:223. Rozbaczylo & Castilla, 1987:176. (Both not *Phasianella mauritiana* Lamarck, 1822 = *Littoraria mauritiana*.)

*Littorina (Austrolittorina) unifasciata fernandezensis* Rosewater, 1970:471–472, pl. 359, figs. 9–12, pl. 361, fig. B (radula) (east shore of Cumberland Bay, Isla Más a Tierra, Juan Fernandez Islands [Chile]; holotype USNM 368900, Rosewater, 1970, pl. 359, figs. 11, 12, Figure 18T herein, seen; 25 paratypes USNM 679256, seen; 11 paratypes DMNH 039221, not seen).

*Nodilittorina (Nodilittorina) fernandezensis*—Reid, 1989a: 99.

*Nodilittorina fernandezensis*—Ramírez & Osorio, 2001:1–13.

**Taxonomic history:** Although Nevill (1885) ascribed the name *penitaria* to W. Wood on the basis of a museum label of unknown provenance, it was apparently never published. The name was rejected as a *nomen nudum* by Rosewater (1970), although arguably the locality alone, as given by Nevill (1885), was sufficient to unambiguously identify the species, since no other member of the family occurs there. This species is abundant on the islands of the Juan Fernández and Desventuradas archipelagos, but these are so remote that it is very rare in museum collections and has scarcely been mentioned in the literature. Odhner (1922) misidentified it as *mauritiana*,

a member of the genus *Littoraria* with a superficially similar greyish white shell. Rosewater (1970) introduced the name as a subspecies of the Australian *N. unifasciata*, correctly recognizing *N. fernandezensis* as a member of the *Austrolittorina* group.

**Diagnosis:** Shell large, whorls rounded, spire profile straight, periphery only slightly angled; aperture finely lirate; spiral sculpture of fine microstriae only; white with broad indistinct blue-grey zone above periphery; single pale basal band within brown aperture. Penial filament small, pointed; small mamilliform gland and large glandular disc borne on projection of base.

**Material examined:** 16 lots (including 15 penes, 3 sperm samples, 5 pallial oviducts, 4 radulae).

**Shell (Figures 18Q–V):** Mature shell height 5.4–21.3 mm. Shape high turbate (H/B = 1.37–1.87, SH = 1.42–2.09); spire whorls rounded, suture distinct; spire profile straight; periphery weakly angled, last whorl slightly flattened. Columella concave, weakly hollowed, slightly pinched at base; sometimes a small eroded parietal area; aperture finely lirate within outer edge. Spiral sculpture of 10–14 weak primary grooves above periphery on early whorls, but by last whorl these become less distinct and only slightly stronger than numerous fine spiral microstriae across whole surface, so shell appears superficially smooth. Periostracum relatively thick, slightly overhanging apertural edge. Protoconch not seen. Color white with broad indistinct zone of blue-grey above periphery; smaller shells often with brownish axial growth marks, or pale brown with basal white band; aperture dark brown, with single pale spiral band at base; columella brown.

**Animal:** Head and sides of foot black. Opercular ratio 0.37–0.60. Penis (Figures 21G–J): filament small, pointed, 0.4–0.5 total length (but available specimens not relaxed), terminal opening of sperm groove; mamilliform gland small, narrow, embedded in enlarged penial glandular disc, together borne on stout projection of base; penis slightly pigmented at base. Posterior 0.25 of prostate is swollen, reddish, closed as a duct; anterior part is thinner and an open groove. Euspermatozoa 66–71  $\mu$ m; paraspermatozoa (Figures 21O, P) round, packed with granules, no visible rod-piece or nucleus, 11–15  $\mu$ m diameter. Pallial oviduct (Figure 21L) with single loop of

Figure 22. Radulae of *Nodilittorina galapagensis* (A, B) and *N. fernandezensis* (C, D); juvenile shells of *N. porcata* (E; note periostracal hairs on basal ribs) and *N. parvicincta* (F); protoconch of *N. modesta* (G); sculpture of *N. modesta* (H; note absence of microsculptural striae). A, B. Punta Estrada, Isla Santa Cruz, Galápagos Islands (BMNH 20001276; two views of radula, flat and at 45°; shell H = 6.9 mm). C, D. Punta San Carlos, Isla Robinson Crusoe (Más a Tierra), Archipiélago de Juan Fernández (BMNH 20001279; two views of radula, flat and at 45°; shell H = 12.8 mm). E. Puerto Ayora, Isla Santa Cruz, Galápagos Islands (BMNH 20001169). F. 7 km N of San José del Cabo, Baja California Sur, Mexico (BMNH 20001179). G, H. Puerto Vallarta, Jalisco, Mexico (BMNH 20001197). Scale bars A–D, G = 50  $\mu$ m; E, F, H = 0.5 mm.

albumen gland followed by large single loop of opaque capsule gland, terminating in reddish translucent portion; copulatory bursa opening at mid-point of straight section, extending back into capsule gland. Spawn not observed; form of oviduct indicates pelagic spawn and likely planktotrophic development.

**Radula (Figures 22C, D):** Relative radular length 2.3–5.0. Rachidian: length/width 1.14–1.27; major cusp elongate, rounded at tip. Lateral and inner marginal: major cusps elongate, rounded or blunt at tip. Outer marginal: 7–9 cusps.

**Habitat:** Volcanic rocks; abundant on rocks and in crevices, in highest intertidal zone, at densities of 65–430 per m<sup>2</sup> (Ramírez & Osorio, 2000). No other littorinids occur with this species.

**Range (Figure 20):** Found only on the Islas Juan Fernández and Islas Desventuradas off the coast of Chile. Range limits: Isla Robinson Crusoe (Isla Más a Tierra), Archipiélago de Juan Fernández (BMNH 20001279); Isla Alejandro Selkirk, Archipiélago de Juan Fernández (BMNH 20001278); Isla San Félix, Islas Desventuradas (BMNH 20001280); Isla San Ambrosio, Islas Desventuradas (BMNH 20001281).

**Remarks:** The large, thick white shells of this species cannot be confused with any other in the eastern Pacific. However, medium and small examples are superficially similar to some pale specimens of *N. araucana* and *N. paytensis*. The former is distinguished by its lack of spiral microstriae, lack of lirae within the aperture, and (when present) stronger primary grooves. *Nodilittorina paytensis* also lacks external microstriae and apertural lirae, has two pale stripes within the aperture, and almost always a pattern of small brown dots. Much more similar, and sometimes indistinguishable except by anatomical characters, are the New Zealand species *N. antipodum* (Philippi) and the Australian *N. unifasciata*; where the locality is unknown, details of penial shape separate these three.

Despite the superficial similarity to *N. araucana* and *N. paytensis*, the present species is not closely related to either of these. Rosewater (1970) recognized its relationships when he described it as a subspecies of the temperate Australian *N. unifasciata* (which he placed in *Littorina*, and included *antipodum* as another subspecies). Likely anatomical synapomorphies of these three species include the short and stout penial filament (although *N. antipodum* and *N. unifasciata* have a slightly subterminal opening of the sperm groove), the narrow mamilliform penial gland often partly embedded in the large penial glandular disc and, most importantly, the additional loop of the egg groove through the opaque capsule gland. This last character state unites a group of littorinids comprising, in addition to these three, *N. cincta* (Quoy & Gaimard) from New Zealand, *N. praetermissa* (May) and *N. acutispira* (E. A. Smith) from southeastern Australia, and

from South Africa *N. africana* (Philippi) and *N. knysnaensis* (Philippi). All eight species are here assigned to *Austrolittorina* Rosewater, 1970 (type species *Littorina unifasciata*). This is provisionally recognized as a subgenus, although in the absence of strong synapomorphies for *Nodilittorina* it is not yet clear that this is correct, and the relationship of *Austrolittorina* with the rest of the genus requires examination with molecular data. Another possible member of the *Austrolittorina* group is the eastern Australian endemic *N. pyramidalis*; this shares the form of the pallial oviduct, but shows an unusual penial shape and is the only one with a nodulose shell. (It may be noted that since *N. pyramidalis* is the type species of *Nodilittorina* (subsequent designation by Abbott, 1954) its relationships and those of *Austrolittorina* have important consequences for the nomenclature of the entire genus; see Remarks on *Nodilittorina*.) As originally constituted, Rosewater's subgenus contained many more species (including e.g., *N. aspera*, *N. peruviana*, smooth-shelled Atlantic *Nodilittorina* species, and *Littoraria tessellata* (Philippi)), and three more species were added later (Ponder & Rosewater, 1979). In revisions of the generic classification of Littorinidae, both Bandel & Kadolsky (1982) and Reid (1989a) considered *Austrolittorina* a synonym of *Nodilittorina*. The subgenus *Austrolittorina* is here used in a restricted sense, and this group shows an austral distribution in temperate and warm temperate latitudes of the Southern Hemisphere.

The shell of *N. fernandezensis* is among the thickest and most solid of all *Nodilittorina* species, but nevertheless the large available samples show an unusually high frequency of scarring and repair on the shell (Figures 18R, T, V). This is more pronounced than in the similar *N. unifasciata*. Whether the shell damage is caused by unusually strong wave action, mobile boulders, or a powerful predator is unknown.

## DISCUSSION

### Intraspecific Shell Variation

Using shell characters alone it would have been impossible to resolve the taxonomy of the three species complexes of *Nodilittorina* in the eastern Pacific. However, having defined the species largely by means of diagnostic penial shapes it was possible to reexamine the confusing range of shell types and thus to discover taxonomically useful characters. The key to interpreting shell variation is that, in each species group, the more obvious shell characters such as size, shape, development of ribs, and color show parallel variation within species, and that it is the more subtle differences in numbers of grooves and color pattern that discriminate between them. Using suitable characters, identification is in fact possible using shells alone in almost all cases.

The degree of intraspecific variation (in shape, sculpture and color in the *N. porcata* group; in sculpture in the



*N. aspera* and *N. modesta* groups and *N. galapagensis*; and in shape and color in *N. araucana*) is remarkable, even among the notoriously polymorphic littorinids. In the well studied temperate genus *Littorina*, extreme intra-specific variability is associated with benthic spawn and a non-planktotrophic mode of development, whereas those species with pelagic egg capsules and planktotrophic development are less variable, at least on a local scale (review by Reid, 1996). The classic explanation for these observations is that the restricted gene flow in non-planktotrophic species permits local genetic adaptation in response to differing selection regimes, resulting in distinctive shell forms or "ecotypes" that are characteristic of different microhabitats on the shore (e.g., Janson, 1983; Seeley, 1986; Johannesson et al., 1993; Trussell, 1997). Controlled rearing has confirmed that some shell traits are indeed heritable (Boulding & Hay, 1993; Johannesson & Johannesson, 1996; Parsons, 1997a). Striking local variation is also known in some planktotrophic littorinids, such as the polymorphism of striated and nodulose forms of *Littorina striata* (Reid, 1996; de Wolf et al., 1997), *Nodilittorina hawaiiensis* (Rosewater & Kadolsky) (Struhsaker, 1968), and *N. australis* (Gray) (Johnson & Black, 1999). Although selection has sometimes been invoked to account for this (Struhsaker, 1968), it would have to be very strong to explain the local-scale variation in the face of high gene flow. Alternative explanations also invoking a genotypic basis might involve non-random larval settlement or some means of limiting dispersal during the pelagic stage. However, there is now increasing evidence from laboratory rearing and field translocation that direct environmental effects on the phenotype play an important role. Examples of ecophenotypic effects include the influences of food availability (mediated via its effect on growth rate) on shell shape (Kemp & Bertness, 1984; Boulding & Hay, 1993; Johnson & Black, 1998) and shell sculpture (Boulding et al., 1993), and the influence of crab predators and water temperature on shell thickness (Trussell, 1996, 2000). Although these demonstrations mostly involve non-planktotrophic littorinids, ecophenotypic effects have also been invoked to explain cases of local variation that are correlated with microhabitat in planktotrophic species (Chapman, 1995; Reid, 1996; Johnson & Black, 1999). Indeed it is argued that in widely dispersed species occupying a range of habitats, phenotypic plasticity should be a favored strategy (Parsons, 1997b).

The evidence for phenotypic plasticity of shell traits in the eastern Pacific *Nodilittorina* species is largely indirect. In common with all known members of the genus, they produce pelagic egg capsules and undergo planktotrophic development. (Although this has not been confirmed by direct observation of spawn and protoconchs in every species, it is predicted from the universal association of a large capsule gland in the pallial oviduct with planktotrophy in members of the Littorininae; Reid,

1989a.) Assuming that this results in high gene flow, the cases of shell variation between microhabitats on the same shore are difficult to explain except by phenotypic plasticity. The most striking examples are the contrasts between the smooth shells of *N. atrata* and *N. santelenae* from mid-shore rock pools and ribbed or carinate shells among barnacles on rocks close by. A possible explanation might be that faster growth under the more favorable conditions in pools results in a smoother shell (as shown in *Littorina sitkana* Philippi by Boulding et al., 1993). Other examples are the dwarf shells of *N. aspera*, *N. tenuistriata*, and *N. apicina* found in small high-shore rock pools that are smaller, smoother, and more darkly patterned than larger shells on open rock surfaces at the same localities. Occasional individuals can be found in which shell shape and sculpture changes abruptly during the course of growth (Figure 11L), supporting the suggestion of plasticity. Similarly, examples of sudden color change (Figures 1G, M) imply likely environmental effects on shell coloration. Although abrupt color change has been observed following translocation between microhabitats in *Littoraria* species (Reid, 1986), there has been no experimental study of phenotypic plasticity in shell color in other littorinids. Among other gastropods, effects of diet upon shell banding patterns have been reported in the trochid *Austrocochlea* (Underwood & Creese, 1976), whereas in neritids this is influenced by cation ratios (Neumann, 1959) and perhaps by salinity (Gundersen & Minton, 1997).

### Other Taxonomically Useful Characters

In addition to their important role in the initial characterization of species, anatomical characters may be required to confirm otherwise doubtful identifications. The simple feature of pigmentation of the head can be useful. Although most of the species show either the typical *Nodilittorina* pattern of head pigmentation, black with a pair of longitudinal black lines on each tentacle, or alternatively are entirely black, in *N. modesta* there are transverse black lines on the tentacles. This provides a useful character for its distinction from the other species in the *N. modesta* group, *N. conspersa*. Tentacle pattern has also been used as a taxonomic character separating two similar *Littorina* species, *L. scutulata* Gould and *L. plena* Gould (Reid, 1996).

As is well known in the Littorinidae, penial shape is the most useful of the taxonomic characters and has frequently provided the first evidence for the discovery of "sibling" species. Since even allopatric sister-species usually show diagnostic penial differences, it has been suggested that penial shape is part of the "specific mate recognition system" (Paterson, 1985) of *Littorina* (review by Reid, 1996). Although the descriptions of eastern Pacific *Nodilittorina* species largely support this idea, the differences among members of species complexes are of-

ten more subtle than those among closely related groups of *Littorina* (Reid, 1996) or *Littoraria* (Reid, 1986, 1999a). Consequently, penial shape is sometimes not entirely diagnostic as, for example, in the *N. porcata* and *N. aspera* species groups (Figures 3, 13, 14). A similar case is known in the sympatric pair *Littorina saxatilis* (Olivi) and *L. arcana* Hannaford Ellis, in which penial shape shows some overlap (Hannaford Ellis, 1979), although genetic results confirm their species status (review by Reid, 1996). It is likely that the shape of the penis during copulation is different from that in relaxed, fixed specimens (see Bingham, 1972, in *Littoraria irrorata* (Say)), perhaps aiding species recognition, or alternatively other unknown recognition cues may be important in *Nodilittorina*. As in *Littoraria* (Reid, 1986), though not in *Littorina* (Reid, 1996), the paraspermatozoa often show marked differences between *Nodilittorina* species, even within species groups (e.g., *N. aspera* group, Figure 15), but the significance of this is unknown.

Among the Littorinidae as a whole, oviduct structure is closely tied to the type of spawn and larval development (Reid, 1989a, 1996). The pallial oviducts of the eastern Pacific *Nodilittorina* species are mostly rather uniform, as expected from their similar (whether known or presumed) life histories. Only that of *N. fernandezensis* is strikingly different from the rest, to which it is probably not closely related (as discussed below). There are small differences between the species groups, but these are not useful for identification within these groups. The egg capsules have been described in five of these species; while these are notably different, it remains to be seen whether this will be the case within species groups. Interestingly, the capsule and contained egg are relatively small in the tropical *N. dubiosa* (capsule 140  $\mu\text{m}$  diameter, ovum 40  $\mu\text{m}$  diameter) and *N. atrata* (160  $\mu\text{m}$ , 40  $\mu\text{m}$ ) from Costa Rica, and larger in *N. paytensis* in Ecuador (300  $\mu\text{m}$ , 84  $\mu\text{m}$ ) and in Chilean *N. araucana* (210–256  $\mu\text{m}$ , 68–84  $\mu\text{m}$ ) and *N. peruviana* (336–421  $\mu\text{m}$ , 84–89  $\mu\text{m}$ ). These data are limited, but a similar trend of increasing egg and capsule size in colder water has been documented in *Littorina* (Reid, 1996), although there is no convincing explanation.

Among other gastropods, radulae may provide useful characters for species discrimination, but this is not usually the case among littorinids (e.g., Reid, 1986, 1996), nor is it so among the eastern Pacific *Nodilittorina*. It has recently been claimed that the radulae of some littorinids show phenotypic plasticity according to the substrate on which they graze (Padilla, 1998; Reid & Mak, 1999). *Nodilittorina* species are almost always found on rocks so that potential plasticity is not easily studied, but in a sample of *N. albicarinata* from grasses the radulae did not differ from the normal form. The members of the *N. porcata* group, all of which are relatively small in size, share a similar radular tooth form with pointed cusps, which on the five central teeth in each row are more uniform in

size than the elongated major cusps seen in the remaining species. In species of *Littorina* both juveniles and small adults show relatively pointed cusps; this has been suggested to be an allometric effect (Reid, 1996) which might also account for the pattern in small *Nodilittorina* species. At up to 15 times the length of the shell, the radula of *N. aspera* is the longest yet reported in this family.

### Phylogenetic Relationships

Although anatomical details are available for all the approximately 60 species of *Nodilittorina* worldwide, attempts at cladistic analysis of morphological characters have so far been uninformative (unpublished observations), a result of relative uniformity in some structures and apparent homoplasy in others. The pallial oviduct is similar in most species (with the exception of the *Austrolittorina* group; there is also some variation in the position of the copulatory bursa), connected with the similar pelagic spawn and planktotrophic development throughout the genus. The radula likewise shows little variation (beyond a trend toward narrowing of the rachidian tooth in some species), which may be related to the high-intertidal rock-dwelling habitat of most of these largely tropical species. The penis too is rather uniform, with a single mamilliform gland and glandular disc in most species and only subtle interspecific variations in overall shape, while there is likely homoplasy in loss of glandular elements. Shell shape and sculpture, however, are too variable within species to provide convenient characters for phylogenetic analysis. In contrast, in the probable sister-genus *Littorina* (which occupies a broad latitudinal temperature range, a range of rock and algal substrates, shows a wide diversity of reproductive modes, and much variation in numbers of penial glands) the analysis of anatomical characters has provided a well resolved cladogram, subsequently supported by molecular data (Reid, 1989a; Reid et al., 1996; Reid, 1996). As noted earlier (see Remarks on the genus *Nodilittorina*) it is not even certain that the genus is monophyletic as presently constituted. In *Nodilittorina* it is likely that only molecular data will satisfactorily resolve phylogenetic relationships. Meanwhile, there are some useful groupings based on morphological resemblance rather than formal cladistic analysis.

The most obvious of these in the eastern Pacific is perhaps the *N. aspera* group, in which resemblances are so close that five of the six species have at one time been considered conspecific (see Remarks on *N. aspera* group). This group shares a spirally sculptured shell without nodules, and a striking shell pattern (usually with a more or less developed dark peripheral band), but penis, pallial oviduct, and radula are each of a form common in the genus. Egg capsules have been described only in *N. dubiosa* and *N. paytensis*, and show some similarity in the absence or slight development of spiral rings on the cu-

pola (compare with figures and references in Reid, 1989a; Mak, 1995; Rudman, 1996). There is a possible parallel in the *N. ziczac* complex in the western Atlantic, comprising between five (Reid, 1989a) and seven (Bandel & Kadolsky, 1982) species. These likewise have strongly patterned shells of similar shape and sculpture, but they exhibit a diversity of penial shapes, radulae, and egg capsules and so may not be a natural group. It is possible that shell similarities between the *N. aspera* and *N. ziczac* complexes are convergent since they are not unique in the genus (e.g., *N. punctata* (Gmelin), *N. peruviana*, and some western Pacific species) and there are no apparent anatomical synapomorphies. Since the eastern Pacific and western Atlantic formed a single marine region until the appearance of the Panamanian Isthmus during the Pliocene, some relationship between their modern faunas is to be expected. Nevertheless, the available evidence gives little indication of this.

A second species complex in the eastern Pacific, the *N. porcata* group, is well defined and probably monophyletic, based on the unique or unusual features of the umbilicate shell, strong expression of likely phenotypic plasticity in sculpture, absence of penial glandular disc, twisted tip to the penial filament, flexure in the straight section of pallial oviduct, pointed radular cusps, and similar mid-shore habitat. The small size, umbilicate shell, and mid-shore or rock pool habitat recalls the pair *N. meleagris* and *N. mespillum* in the Atlantic and Caribbean. Rosewater (1981) introduced the subgenus *Fossarilittorina* for these two species (with *N. meleagris* as type species) and added *N. atrata*, *N. porcata*, and *N. albicarinata*. In this case, a relationship with the Atlantic species is supported by the possible synapomorphies of umbilicate shell, absence of penial glandular disc, flexure in the oviduct, and radular cusps (although none of these is individually unique, their combination is not found elsewhere in the genus). Furthermore, paraspermatozoa and egg capsules (known only in *N. atrata* and *N. meleagris*; personal observation) are closely similar. The most significant difference is in the penis which, in the Atlantic species, lacks all glandular projections and has a superficially closed sperm duct (Reid, 1989a). The former condition is found as a rare abnormality in the eastern Pacific species (*N. atrata* and *N. parcipicta*), and the latter is a minor anatomical modification found elsewhere in littorinids, so while these characters are likely synapomorphies of *N. meleagris* and *N. mespillum* they do not preclude a sister-group relationship with the *N. porcata* complex.

The two species of the *N. modesta* group are undoubtedly sister-species, sharing almost identical shells, a unique simple and vermiform penis, a unique synapomorphy of projection of the renal oviduct into the spiral of the albumen gland, and similar extra denticles on the rachidian tooth. The relationships of this clade are nevertheless obscure. As a result of a cladistic analysis of the Littorini-

dae, Reid (1989a) tentatively placed *N. modesta* (then considered a single species) together with *N. meleagris* and *N. mespillum* in the subgenus *Fossarilittorina*. However, the only synapomorphy was the absence of mamilliform penial glands which, as suggested above, is weak and liable to convergence, since mamilliform glands are readily lost (also noted in *Littorina*, Reid, 1996; and *Peasiella*, Reid, 1989b).

*Nodilittorina fernandezensis*, endemic to the oceanic islands off Chile, is the only one of the eastern Pacific *Nodilittorina* species to show a clear relationship with a group of species to the west. As discussed in the Remarks on the species, it is placed in the subgenus *Austrolittorina* (type species *N. unifasciata*) together with at least seven species from southern Australia, New Zealand, and South Africa. This subgenus is defined by the synapomorphy of the additional loop of the egg groove through the opaque capsule gland (elsewhere in *Nodilittorina* shared only with *N. pyramidalis*, a species of uncertain relationships but possibly belonging to the same clade) and additionally characterized by similarities of penial shape and weak shell color pattern.

The remaining eastern Pacific *Nodilittorina* species cannot at present be convincingly linked with other groups within the genus (see Remarks on *N. araucana*, *N. peruviana*, and *N. galapagensis*).

With the exception of *Austrolittorina*, subgeneric names have not been used here for the tentative groupings suggested. In an earlier review of the genus, Bandel & Kadolsky (1982) remarked on the pervasive homoplasy and likewise did not designate subgeneric groups. In the only formal cladistic analysis, Reid (1989a) accepted three subgenera *Fossarilittorina*, *Echinolittorina*, and *Nodilittorina*. The doubt surrounding the first has been discussed earlier. The latter two were distinguished only by the relative position of the copulatory bursa in the pallial oviduct, but new observations (presented here and unpublished) suggest that this distinction is not clear cut. Molecular data are urgently required to address these phylogenetic questions.

## Distribution Patterns and Faunal Provinces

The attempt to define faunal provinces is an unfashionable part of marine zoogeography, having been superseded by studies of regional biodiversity and the phylogenetic approach to historical biogeography. Nevertheless, the twin concepts of faunal provinces and the boundaries between them are heuristically useful, serving as a framework for distributional data, highlighting dispersal processes, and influencing sampling for systematic and genetic studies. Provinces have been defined either by an arbitrary level of endemism (10% was taken by Briggs, 1974) or by coincidence of many end-points of ranges. However, the distributions of taxonomic groups respond to environmental barriers in different ways, according to

their biogeographic history, dispersal capabilities, habitat requirements, and physiological tolerances. Therefore, it is not useful to seek a universal classification of marine faunal provinces, except in the broadest terms.

The recognition of marine faunal provinces in the eastern Pacific Ocean has a long and complex history. Since their distributions are relatively well known, studies of mollusks have played an important part (e.g., Carpenter, 1857b; Dall, 1909; Newell, 1948; Olsson, 1961; Valentine, 1966; Bernard et al., 1991). There is general agreement that the tropical region extends from the Gulf of California south to northernmost Peru, including the oceanic islands (Islas Revillagigedo, Isla del Coco, Isla de Malpelo, and Galápagos). This has been named the Eastern Pacific Zoogeographic Region (Briggs, 1974), but is now commonly referred to as the Tropical Eastern Pacific or TEP (Hastings, 2000). Although Briggs (1974) classified the Gulf of California (Sea of Cortez) as part of the warm-temperate region to the north, its chief affinities lie with the TEP, and its designation as tropical is not now disputed (Brusca, 1980; Hastings, 2000). The northern limit of the TEP on the Pacific coast of Baja California is set by the influence of the cold southerly California Current. However, this limit is not sharply defined, since this coast is complex, with lagoons and bays providing refugia for tropical species, whereas exposed coasts and upwelling zones harbor a temperate fauna. This is best regarded as a transitional zone between the TEP and warm-temperate Californian Province, lying approximately between Punta Eugenia and Bahía Magdalena (Brusca & Wallerstein, 1979; Brusca, 1980) or extending farther south to Cabo San Lucas (Bernard et al., 1991). The southern limit of the TEP also corresponds to a steep temperature gradient, where the cold northerly Peru (Humboldt) Current sweeps offshore, between the Golfo de Guayaquil and Punta Aguja (3–6°S) (Keen, 1971; Brusca & Wallerstein, 1979; Bernard et al., 1991; Hastings, 2000). To the south the Peruvian Province (or Peru-Chilean, Briggs, 1974) is of warm-temperate character and extends down the coast of Peru and Chile to merge with the Magellanic Province in a broad transitional zone between 30–46°S (Viviani, 1979; Brattström & Johansen, 1983; although Bernard et al., 1991, combined these as a single Chilean Province).

The provincial classification of the oceanic islands of the eastern Pacific is problematic, since the faunas are generally impoverished relative to the mainland and are often poorly studied. In addition they may include a proportion of rare species that are immigrants from either the Indo-West Pacific or from the mainland, and that do not become established. The classification of the tropical islands is discussed below. Of particular interest are the warm-temperate oceanic islands off Chile, the Islas Desventuradas, and Islas Juan Fernández. Their molluscan faunas are little known (Odhner, 1922; Rozbaczylo & Castilla, 1987; Bernard et al., 1991), but some species are

shared with the Peruvian Province. However, in a list by Rozbaczylo & Castilla (1987) 72% of the 39 recorded mollusks (excluding cephalopods) were given as endemic. In a later compilation of the bivalves, 26% of the 31 species from the Juan Fernández Archipelago were recorded as endemic, none was shared with the Indo-West Pacific, only three were shared with the still more poorly known Islas Desventuradas, and no endemics were noted on those islands (Bernard et al., 1991). Although these two island groups are only 600 km from the mainland and 800 km apart, the islands appear to be isolated from the continent by the Peru Current flowing northward parallel to the Chilean coast (Bernard et al., 1991). The single littorinid found there, *N. fernandezensis*, is endemic and appears to be conspecific on the two island groups. As noted earlier, its relationships are undoubtedly with a southern-temperate group from Australia, New Zealand, and South Africa (subgenus *Austrolittorina*). Similar trans-Pacific relationships of mollusks at subtropical and warm-temperate latitudes (for example, of Islas Juan Fernández and Isla de Pascua with Australia and New Zealand) have been noted before (Rehder, 1980; Lindberg & Hickman, 1986; Bernard et al., 1991). The distances are too great for transport of pelagic larvae (except teleplanic forms) in oceanic currents, but rafting of adults has been suggested for a trans-Pacific oyster (Ó Foighil et al., 1999). Alternatively, trans-Pacific dispersal of shallow-water species may have been facilitated by the presence of little-known or uncharted reefs in these latitudes in the South Pacific Ocean; at times of low sea level these may have emerged as islands to act as stepping stones for littoral species (P. Bouchet, personal communication). In a recent list of 51 fishes of the Juan Fernández Islands, Pequeño & Sáez (2000) found that 25.5% were endemic, 29.4% shared only with the Islas Desventuradas, and that slightly more species were shared with Pacific islands to the west than with the mainland to the east (19.6% compared with 15.6%). It seems appropriate that the Islas Juan Fernández and Desventuradas should together be included in a distinct Juan Fernández Province, as proposed by Briggs (1974), although whether this is classified as part of the warm-temperate region of the South American mainland (Briggs, 1974) or of the tropical Indo-West Pacific region (Pequeño & Sáez, 2000) is debated.

Opinions about the subdivision of the TEP region into smaller faunal provinces are diverse and dependent upon the group studied. Molluscan workers have, with few exceptions, emphasized the faunal uniformity of the TEP (named the "Panamic Province" in molluscan texts) and have not identified distributional boundaries within it (Dall, 1909; Keen, 1958, 1971; Olsson, 1961; Bernard et al., 1991; Emerson & Chaney, 1995; Roy et al., 1998). This is so even when considering the molluscan fauna of the Galápagos Islands with endemism estimated as 18–23% (Finet, 1991; Kay, 1991). However, Keen (1958) remarked on Panamic "subprovinces" in the northern

Gulf of California and Gulf of Panama, and Vermeij (1991) suggested that the Mexican coast and Gulf of California have acted as refuges from extinction for mollusks that were formerly more widespread within the TEP. Working with echinoderms, Maluf (1988) found high overall faunal similarity within the TEP from Cabo San Lucas to Peru, but recognized the Gulf of California (Cortez Province) as distinct (based on species shared with the Californian Province, and despite low endemism of only 2%) and also the Galápagos Province (endemism 16%). Using decapods, Correa-Sandoval & Rodríguez-Cortés (1998) accepted a Cortez Province with 24% endemism, distinct from Mexican and Panamic Provinces to the south, contrary to an earlier study in which Cortez and Mexican Provinces were united (Hendrickx, 1992). Based on analysis of the depauperate zooxanthellate coral fauna of the TEP, Glynn & Ault (2000) found similarities among the Islas Revillagigedo, Gulf of California, and southern Mexico, suggesting a provincial difference from the group of southern localities (Central America, Ecuador, Galápagos). However, it has been studies of shore fishes that have led to the clearest subdivision of the TEP. Although with differences of detail, most workers have divided the region into four provinces: Cortez, Mexican, Panamic, and Galápagos (Hubbs, 1952; Briggs, 1955, 1974; Springer, 1958; Walker, 1960; Hastings, 2000), separated mainly by gaps of open ocean and of inhospitable coastline without rocky substrates. The limits of these provinces can be defined as follows (where authors disagree, the boundaries have been selected for maximum agreement with the distributions of *Nodilittorina* reported here; see Figure 23). The Cortez Province includes the entire Gulf of California as far south as La Paz on the eastern coast of Baja California (Hubbs, 1952; Briggs, 1974) and Topolobampo (Sinaloa) on the mainland (Briggs, 1974; Hastings, 2000). The northern boundary of the Mexican Province is disputed; Hastings (2000) restricted this province to the mainland south of Mazatlán, while extending the Cortez Province around the tip of Baja California to the junction with the Californian Province. Here, however, the southwestern coast of Baja California (Punta Eugenia to La Paz) is included with the Mexican mainland south of Mazatlán (Hubbs, 1952; Springer, 1958; Walker, 1960; Briggs, 1974). The southern limit of the Mexican Province is near Salina Cruz in the Golfo de Tehuantepec (Southern Mexico) (Briggs, 1955, 1974; Springer, 1958; Hastings, 2000). Here, the Islas Revillagigedo are classified in the Mexican Province, although included in the Panamic Province by Briggs (1974). The Panamic Province (Panamanian of Briggs, 1974) is restricted to the region south of the Golfo de Fonseca (between El Salvador and Nicaragua) (Springer, 1958; Hastings, 2000) and includes Isla del Coco (Cocos Island) and Isla de Malpelo.

The distributions of *Nodilittorina* species and, for comparison, of *Littoraria* species (from Reid, 1999a) are sum-

marized in Table 4. There is a close correspondence with the faunal provinces as defined on the basis of shore fishes. Of the 18 species of *Nodilittorina*, only four extend their distributions (with apparently self-sustaining populations) through large parts of two adjacent provinces (*N. atrata* and *N. conspersa* in Panamic and Galápagos; *N. albicarinata* in Cortez and Mexican; *N. apicina* in Mexican and Panamic). For these littorinids, the barriers between the provinces are evidently remarkably effective. Oceanographic conditions clearly play some part. The steep temperature gradients at the northern and southern boundaries of the TEP have been mentioned, but temperature limitation is probably not significant within the TEP where temperatures exceed 20–25°C throughout the year (Bernard et al., 1991; Correa-Sandoval & Rodríguez-Cortés, 1998). During El Niño events the latitudinal extent of the TEP widens, which may account for occasional records of Panamic mollusks beyond their normal limits in northern Peru (Paredes et al., 1998). The Galápagos Islands are isolated from the mainland of Ecuador by 1000 km of open ocean, although under the influence of the Peruvian Current and of the Panama Current (from January to April) (Finet, 1991). Within the TEP the major currents are the northward Costa Rica Current (stronger in the summer, when it reaches the Gulf of California), the Panama Bight Gyre and the Panama Current (Bernard et al., 1991; Correa-Sandoval & Rodríguez-Cortés, 1998; Glynn & Ault, 2000) but, while significant for dispersal, these are not obviously connected with provincial boundaries. The influence of oceanographic conditions related to productivity, upwelling, and freshwater inflow are not understood. The distinction between “oceanic” and “continental” distributions among littorinids and other mollusks has often been noted (Reid, 1986, 1989b, 1999a) and may in some way be connected with the high productivity, turbidity, and runoff on continental margins. It may therefore be significant that the Panamic Province includes three areas of upwelling (in the Gulfs of Tehuantepec, Papagayo, and Panama) and has by far the highest freshwater input in the TEP, resulting in high algal productivity and turbidity (Glynn & Ault, 2000; Oceanic Primary Productivity Study, Rutgers University), thus providing a typically “continental” habitat for shallow-water mollusks. The Gulf of California is also an area of high oceanic productivity, whereas the Pacific coast of Baja California and most of the Mexican Province (with the exception of a periodic upwelling off the coast of Jalisco) provide typically “oceanic” conditions of low productivity and clear water (Santamaría-del-Angel et al., 1994; Barnard et al., 1999; Glynn & Ault, 2000; Oceanic Primary Productivity Study, Rutgers University). However, by far the most important determinant of provincial boundaries along the contiguous TEP coastline appears to be simply the availability of suitable intertidal habitat.

As recognized by workers on shore fishes that, like *Nodilittorina* species, require rocky substrate, the Cortez

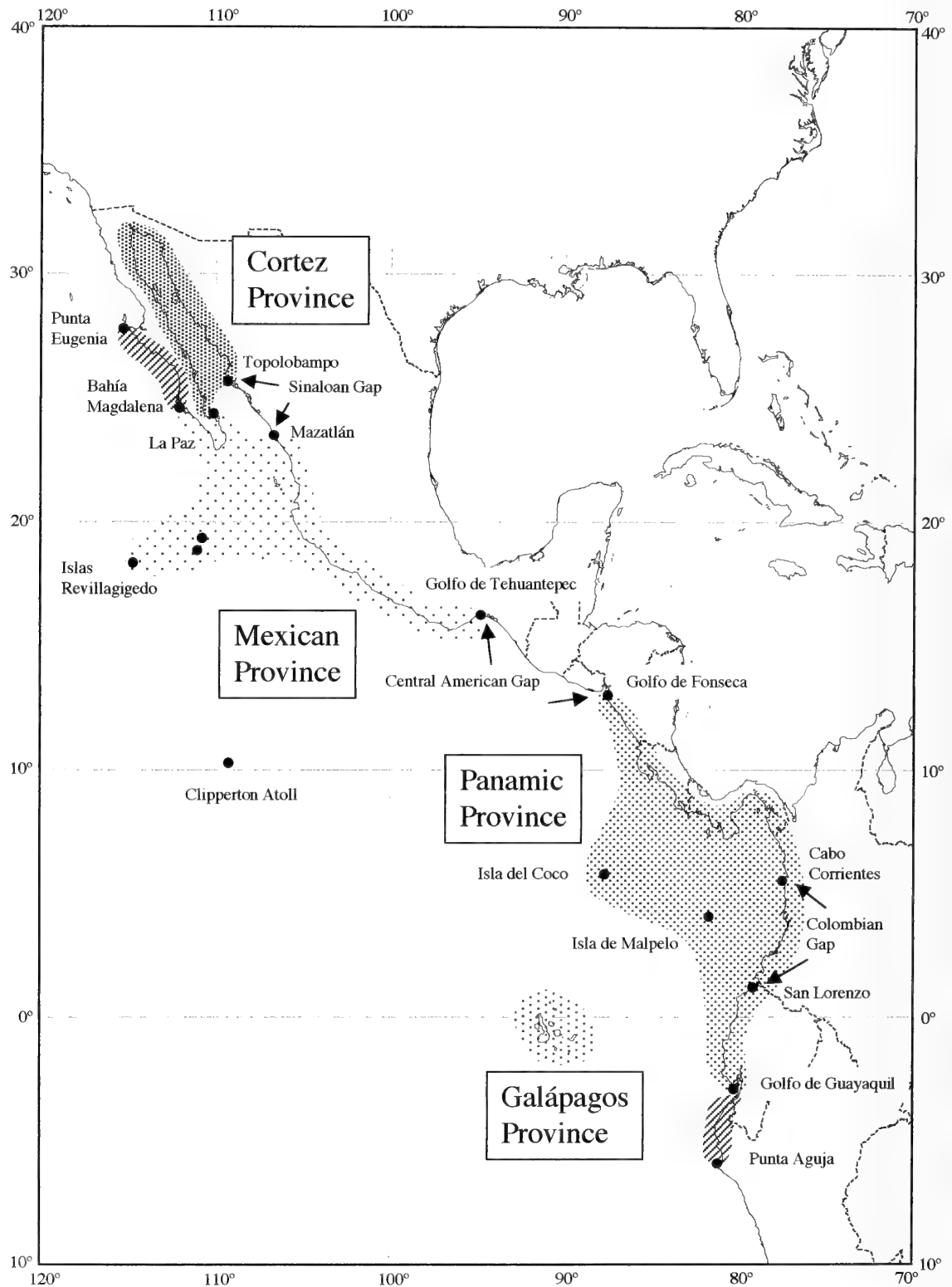


Figure 23. Faunal provinces of the Tropical Eastern Pacific Region (TEP), based on distribution of shallow-water fauna of rocky substrates, principally fish and *Nodilittorina* species (modified from Springer, 1958; Briggs, 1974; Hastings, 2000). Cross-hatched areas are transitional zones between TEP and (to the north) the Californian Province and (to the south) the Peruvian Province.



Table 4

Distributional ranges of Littorinidae (*Nodilittorina* and, from Reid, 1999, *Littoraria*) from Baja California to Chile, listed by marine faunal provinces (Figure 23; see text for definitions). For *Nodilittorina*, "species groups" are tentatively suggested as possible monophyletic groups, based on likely synapomorphies and overall similarity. Species are listed in the provinces in which they have all or a significant part (i.e., likely self-sustaining populations) of their distributions. Occasional rare records are indicated by +.

Species groups	Californian	Cortez	Mexican	Panamic	Galápagos	Peruvian	Juan Fernández
<i>N. atrata</i>				<i>N. atrata</i>	<i>N. atrata</i>		
<i>N. porcata</i>					<i>N. porcata</i>		
<i>N. santelenae</i>				<i>N. santelenae</i>			
<i>N. fuscolineata</i>				<i>N. fuscolineata</i>			
<i>N. parcipicta</i>		+	<i>N. parcipicta</i>				
<i>N. albicarinata</i>	+	<i>N. albicarinata</i>	<i>N. albicarinata</i>				
<i>N. modesta</i>		+	<i>N. modesta</i>	+			
<i>N. conspersa</i>			+	<i>N. conspersa</i>	<i>N. conspersa</i>		
<i>N. aspera</i>		+	<i>N. aspera</i>	+			
<i>N. tenuistriata</i>				<i>N. tenuistriata</i>			
<i>N. dubiosa</i>				<i>N. dubiosa</i>	+		
<i>N. apicina</i>	+		<i>N. apicina</i>	<i>N. apicina</i>			
<i>N. penicillata</i>		<i>N. penicillata</i>	+				
<i>N. paytensis</i>				<i>N. paytensis</i>		+	
<i>N. araucana</i>						<i>N. araucana</i>	
<i>N. peruviana</i>						<i>N. peruviana</i>	
<i>N. galapagensis</i>					<i>N. galapagensis</i>		
<i>N. fernandezensis</i>							<i>N. fernandezensis</i>
<i>L. pintado pullata</i>			<i>L. pintado pullata</i>	<i>L. pintado pullata</i>			
<i>L. varia</i>				<i>L. varia</i>			
<i>L. zebra</i>				<i>L. zebra</i>			
<i>L. variegata</i>		<i>L. variegata</i>	<i>L. variegata</i>	<i>L. variegata</i>			
<i>L. rosewateri</i>		<i>L. rosewateri</i>	<i>L. rosewateri</i>	<i>L. rosewateri</i>			
<i>L. aberrans</i>				<i>L. aberrans</i>			

and Mexican Provinces are separated by a stretch of predominantly muddy coastline with mangroves and deltas that extends for 700 km from Guaymas to Mazatlán (Briggs, 1955, 1974; Springer, 1958). An isolated rock outcrop occurs at Topolobampo (Sinaloa), and this is now taken as the southern limit of the Cortez Province for shore fishes, separated from the Mexican Province by the "Sinaloan Gap" of 370 km (Hastings, 2000; Figure 23). A similar barrier, the Central American Gap, separates the Mexican and Panamic Provinces, consisting of over 1200 km of sand, mud, and mangrove lagoons between the Golfo de Tehuantepec and the Golfo de Fonseca (Springer, 1958; Briggs, 1974; Hastings, 2000; Figure 23). Likely stepping stones are found in El Salvador at Los Cobanos and La Libertad, where, respectively, *N. atrata* and *N. apicina* have been found (see also Glynn & Ault, 2000). The boundary between the Cortez and Mexican Provinces in the vicinity of La Paz, Baja California, cannot be explained so easily. Rocky shores are more or less contin-

uous, but perhaps the greater wave exposure and lower oceanic productivity in southeastern Baja California are significant; these conditions are more similar to the mainland coast of the Mexican Province than to the Gulf of California. Those *Nodilittorina* species restricted to the Mexican Province are characteristic of wave-exposed coasts (*N. parcipicta*, *N. modesta*, *N. aspera*) and those of the Cortez Province of sheltered shores (*N. albicarinata*) or a range of exposure (*N. penicillata*).

In addition to the Sinaloan and Central American Gaps, there is another large expanse of sedimentary shore and mangroves, extending more than 500 km between Cabo Corrientes (Colombia) and San Lorenzo (Ecuador). The biogeographic implications do not appear to have been mentioned previously and the area can be termed the Colombian Gap (Figure 23). There is some evidence from the distributions of *Nodilittorina* species that this gap also presents a barrier to dispersal. Of the six species occurring commonly in the Panamic Province north of this gap,



only four are also common south of it in Ecuador (*N. atrata*, *N. conspersa*, *N. tenuistriata*, *N. apicina*). Of the other two, *N. fuscolineata* is likely a chance immigrant in Ecuador, whereas *N. dubiosa* has not been recorded south of Isla Gorgona, which is a stepping stone of rocky shore within the gap. Conversely, *N. santelenae* is endemic to Ecuador and northern Peru. The status of *N. paytensis* is uncertain; it is abundant in Ecuador and northern Peru, but there are only three records from Colombia and Costa Rica, only one of which was of a large population; it may therefore be another species virtually endemic to the tropical region south of the Colombian Gap. This gap is evidently a less effective barrier for littorinids than the two farther north, perhaps owing to the presence of stepping stones, but is still significant for some. These data as yet provide little evidence upon which to subdivide the Panamic Province, although the possibility should be considered when the poorly known Ecuadorean fauna is studied. It should also be noted that as natural coastlines are altered by clearing of mangroves and building of marine structures to act as artificial stepping stones, these three gaps in the rocky-shore fauna of the TEP may become less effective, permitting permanent range extensions into adjacent provinces (Glynn & Ault, 2000).

While the correspondence between littorinid distributions and the provinces of the TEP is striking, the habitat and water gaps delimiting the provinces clearly do not present impassable barriers. In fact there are eight known cases of species that are recorded as rare arrivals in adjacent provinces (Table 4; not including two extensions into the temperate Californian and Peruvian Provinces). The dispersal capabilities of tropical littorinids are not known in detail. Only a single species, *N. hawaiiensis*, has been successfully reared in the laboratory, taking on average 24 days from spawning to metamorphosis at 25°C (Struhsaker & Costlow, 1968, as "*Littorina picta*"). From similarities in oviduct structure and protoconch throughout the genus, planktotrophic development in the *Nodilittorina* species of the TEP is predicted to be similar to that of this Indo-Pacific species. At this rate of development, a moderate current speed of only 40 km per day would be sufficient to transport pelagic eggs and larvae for 1000 km. Therefore it is not remarkable that mainland species reach the Galápagos Islands, or that species can span the Central American Gap. More surprising is that these barriers are so effective and that immigrants do not become established. While environmental conditions or competitive effects might be invoked, it should also be noted that establishment of self-sustaining populations of planktotrophic-developing species at a large distance from the source population is difficult, since pelagic eggs and larvae are swept away from founding individuals and settle only at very low density (Johannesson, 1988). Thus wide habitat gaps may indeed be effective barriers to colonization, although not to occasional immigration. In as-

sessing the causes of provinciality within the TEP, it is interesting to compare the distributions of *Nodilittorina* with those of the only other native littorinid genus, *Littoraria* (Table 4; Reid, 1999a). Of the six endemic *Littoraria* species, only one (*L. pintado pullata* (Carpenter)) occurs on rocky shores and this is restricted to the Mexican Province (the southern tip of Baja California and Mexican mainland), but also including Clipperton Atoll (at the boundary between the Indo-West Pacific and TEP) and Isla del Coco (classified as part of the Panamic Province). However, the five remaining species inhabit mangrove trees and (in some cases) salt marsh vegetation. Of these five, three are strictly Panamic, whereas two (*L. variegata* (Souleyet) and *L. rosewateri*) extend the length of the TEP from the Gulf of California to Peru. These species are more widespread than any of the rocky-shore *Nodilittorina* species, perhaps because the mangrove habitat is more continuous (and provides more opportunities for dispersal by rafting). For these mangrove-associated species the significant barrier is the expanse of rocky shore without open-coast mangrove habitats (Glynn & Ault, 2000) along almost the entire mainland coast of the Mexican Province (Reid, 1999a). Significant barriers to dispersal, and hence the designation of "provincial boundaries," can therefore differ among ecological (and taxonomic) groups of animals.

To the west of the TEP lies the great expanse (at least 5400 km) of open ocean that constitutes the Eastern Pacific Barrier, the most effective oceanic barrier to the dispersal of shallow-water fauna in the world's oceans (Grigg & Hey, 1992). Even this barrier is not impermeable to animals with sufficiently long pelagic stages and it acts as a largely unidirectional (west to east) filter bridge (Glynn & Ault, 2000). Recently, three littorinid species from the Indo-West Pacific (IWP) Province have been recorded from the TEP for the first time, from Clipperton Atoll, Isla del Coco, and Costa Rica (Reid & Kaiser, 2001). So far, no trans-Pacific *Nodilittorina* species have been found in the TEP. The most isolated of the oceanic islands in the TEP is Clipperton Atoll, with the highest representation of IWP fauna in the TEP (Emerson, 1991; Robertson & Allen, 1996; Glynn & Ault, 2000). Of the TEP *Nodilittorina* species only *N. modesta* is found at Clipperton Atoll, where it is an occasional immigrant.

This review of provinciality in the rocky-shore fauna of the TEP holds potentially important implications for systematic malacology in the region. The prevailing concept in the malacological literature of a uniform "Panamic Province" from the Gulf of California to northern Peru (i.e., equivalent to the TEP region) is based largely on two influential studies of bivalves (Olsson, 1961; Bernard et al., 1991). Since bivalves are predominantly a subtidal, soft-bottom group, this may explain why the provinciality of the TEP has not been more widely noticed previously. As revisionary work progresses on the

shallow-water gastropods of hard substrates, it is likely that genera in addition to *Nodilittorina* will show a more marked provincial diversity than is currently recognized. Already Vermeij (2001) has indicated examples in the genera *Neorapana*, *Stramonita*, and *Nerita*. Even in some infaunal bivalve genera, careful systematic work has revealed provincial endemics as well as genuinely widespread species (Coan, 1983; Roopnarine, 1996). In future, when sampling supposedly widespread species from the TEP for systematic and genetic purposes, samples should be included from the four TEP provinces described here (Figure 23) and long known in other animal groups.

### Historical Biogeography and Speciation

In the absence of both a rigorous phylogenetic hypothesis and a fossil record, discussion of historical biogeography and patterns of speciation can only be speculative. The Pliocene history of Central America is dominated by the uplift and (at 3.1–2.8 Ma) final closure of the Isthmus of Panama (Coates & Obando, 1996). This vicariant event separated the biota of the TEP and tropical western Atlantic and had profound evolutionary consequences, being followed by a marked impoverishment of the tropical American marine fauna. The causes and timing of the extinctions are still debated, but they had a more pronounced effect in the Caribbean. As a result, during the later Pliocene many of the taxa formerly widespread in tropical America became restricted to the Pacific side of the isthmus, far outnumbering those that survived only on the Atlantic side (Vermeij & Petuch, 1986; Vermeij, 1991, 1993). Nevertheless, overall molluscan diversity has remained comparable in both oceans, perhaps because extinction in the western Atlantic was balanced by speciation and immigration (Allmon et al., 1993, 1996; Jackson et al., 1993). However, when inter-oceanic comparisons have been made within single molluscan clades with a good fossil record, they have revealed both higher extinction in the western Atlantic and higher diversification in the TEP, resulting in the modern higher diversity of the latter (e.g., chionine Veneridae, Roopnarine, 1996; *Strombina*, Jackson et al., 1996; *Thais*-like muricids and others, Vermeij, 2001). Among littorinids, there is evidence of higher modern diversity in the TEP than in the western Atlantic within a clade of mangrove-associated members of *Littoraria*, but in the absence of a fossil record this cannot yet be ascribed to differential diversification or extinction (Reid, 1999a).

Against this background, the perceived higher diversity of the genus *Nodilittorina* in the Caribbean than in the TEP under previous classifications of the group was surprising. The most recent listing (Reid, 1989a) gave five species in the TEP (*N. porcata*, *N. albicarinata*, *N. modesta*, *N. aspera*, *N. galapagensis*) and eight (*N. meleagris*, *N. mespillum*, *N. angustior* (Mörch), *N. dilatata*, *N. interrupta*, *N. riisei*, *N. tuberculata*, *N. ziczac*) in the Ca-

ribbean. The revision of *Nodilittorina* species in the TEP shows that this is not in fact the case, the recognized total for the entire TEP region being 15 species (Table 4). Despite the relatively recent separation of the TEP and Caribbean faunas, the possible phylogenetic relationships between *Nodilittorina* species on either side of the Isthmus remain obscure. As suggested earlier, the *N. porcata* group may perhaps be a sister-radiation to the Caribbean pair, *N. meleagris* and *N. mespillum*, and the *N. aspera* group shows some similarity to the *N. ziczac* group in the Caribbean. Nevertheless, the lack of clear trans-isthmian relationships implies that significant diversification and/or extinction in the two regions has taken place since their separation.

Preliminary studies of the distributions of sister-species pairs in Littorinidae suggest that the prevailing mode of speciation has been allopatric (Reid, 1994, 1996). The distributional data for *Nodilittorina* in the eastern Pacific support this conclusion, since species pairs and triplets that are likely most closely related are largely allopatric (Table 4). Whether speciation in these cases has proceeded by vicariance of an originally more extensive range by imposition of a barrier to gene flow, or by dispersal across a pre-existing barrier (founder or peripatric speciation) cannot yet be ascertained (except in the case of Galápagos and Juan Fernández endemics, for which only founder speciation is possible). If, as argued earlier, habitat gaps are the main determinants of range limits for these species in the TEP, then knowledge of the age of the coastal landforms will be important. The observation that dispersal across these barriers is relatively frequent might suggest that founder events have played a part.

This diverse group of rocky-shore gastropods, with precisely known geographical distributions, could provide a model system for the study of speciation in the TEP. First, however, a robust phylogenetic hypothesis is required and, for this, molecular data are now being sought.

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## NOTES, INFORMATION & NEWS

### A Useful Marker for the Study of Neural Development in Cephalopods

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In cephalopods, there is no suitable marker that visualizes three-dimensional neural patterns in the preserved embryos. Development of the nervous system has been studied using conventional histological techniques (Meister, 1972; Marquis, 1989). Silver impregnation and methylene blue staining have been used in anatomical studies of the nervous system in cephalopods (Stephens, 1971) but the former are applicable only to late embryonic stages (Martin, 1977) and the latter, only to living neurons. Cobalt backfilling (Budelmann & Young, 1987) and DiI injection (Robertson et al., 1993) only label local neuronal patterns. We tried whole mount immunostaining of cephalopod embryos and hatchlings with commercially obtainable monoclonal antibodies and found acetylated  $\alpha$ -tubulin a suitable immunohistochemical marker to visualize the overall pattern of developing neurons.

We used four sepiids, *Idiosepius paradoxus* Ortmann, 1881; *Euprymna morsei* (Verrill, 1881); *Sepia lycidas* Gray, 1849; *Sepiella japonica* Sasaki, 1929; two teuthoids, *Loliolus japonica*, Hoyle, 1885; *Todarodes pacificus* Steenstrup, 1880; and an octopod, *Octopus ocellatus*

Gray, 1849 (Octopoda). Dechorionated embryos at various stages of neurogenesis and hatchlings were fixed in 4% paraformaldehyde (PFA) dissolved in phosphate buffered saline (pH 7.6) (PBS) for 12–24 hr at 4°C. Samples were washed with PBS, dehydrated in a methanol series, and stored in 80% methanol at –20°C. Some samples were also fixed in Bouin's solution/seawater, dehydrated with an ethanol series, and stored in 70% ethanol at room temperature. The stored samples to be immunostained were placed as a whole, or after dissection into a few pieces, in ice-cold 50% dimethyl sulfoxide (DMSO)/methanol with 10% hydrogen peroxide for 5 min. They were incubated for 30 min at 4°C in the DMSO solution with 1% Triton X-100, washed with Tris-buffered saline (TST; 20 mM Tris-HCl, pH 8.0, 150mM NaCl, 0.1% Triton X-100) containing 5% DMSO, and blocked with 5% non-fat dry milk (TSTM) overnight at 4°C. The specimens were incubated with anti-acetylated  $\alpha$ -tubulin antibody (Sigma) diluted 1:750–1000 in TSTM for 2–4 d at 4°C (Gianni & Fuller, 1985). After being washed with TSTM, some samples were incubated in pre-diluted goat anti-mouse antibody conjugated to peroxidase (Envision+, DAKO) for 12–24 hr at room temperature, washed with TSTM, immersed in ice-cold 3,3'-diaminobenzidine (DAB) (1 mg/ml TST) for 1 hr, and reacted by adding hydrogen peroxide (0.01%) for 5–20 min in the dark. The other samples were stained with ABC high-HRP immunostaining kit (TOYOBO) according to the standard protocol.

Anti-acetylated  $\alpha$ -tubulin clearly stained peripheral nerve fibers as well as neuropiles in the brain (Figure 1A). It also stained the epidermal cilia, lateral lines, ectodermal photosensitive vesicles, Kölliker's canals of the statocysts, and olfactory organs. The antibody recognized neurons even in Bouin-fixed specimens that had been stored for 3 yr in ethanol, though not always consistently. As for the secondary antibodies, Envision+ (DAKO) was slightly more effective than ABC high HRP (TOYOBO) kit. The intensity and the extent of visualization depended on the limit of penetration of the antibodies. In small specimens, such as the embryos and hatchlings of *I. paradoxus* and the embryos of *E. morsei*, all neuronal elements, i.e., peripheral nerves and neuropiles in the brain, were observable in the samples mounted as a whole (Figure 1B). In larger specimens, such as *O. ocellatus*, *S. lycidas*, *S. japonica*, and *L. japonica*, dissection was necessary before immunostaining permitted visualization of the neuropiles in the deep portion of the brain and all the peripheral nerve fibers in the body. We tested two other monoclonal antibodies, anti-neurofilament 200 (Sigma)

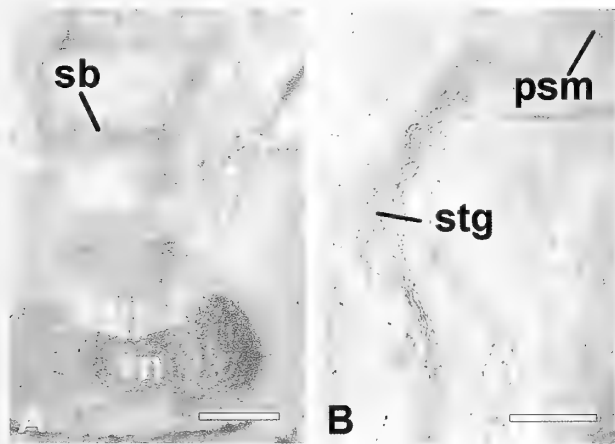


Figure 1. Neuropiles in whole-mount of specimens immunostained with acetylated  $\alpha$ -tubulin antibody. A. The vertical lobe (vn) and the superior buccal lobe (sb) in a late embryo of *Sepia lycidas*. The cranial cartilage is removed. Scale bar = 300  $\mu$ m. B. The stellate ganglion (stg) and the posterior subesophageal mass (psm) in an *Idiosepius paradoxus* hatchling. Scale bar = 30  $\mu$ m.

and anti-HRP (Sigma) (Jan & Jan 1982), but they did not recognize any neuronal elements.

**Acknowledgments.** We thank Mr. W. Godo and Dr. T. Akiyama for their help in collecting cephalopods, and Ms. M. Yoshioka, Dr. S. Segawa, Dr. K. Fujita, and Mr. K. Kidokoro for supplying some cephalopod samples. Thanks are also due to Dr. Sv. Bolzky for his kind help in preparing the manuscript.

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#### *Crepidula dilatata* Lamarck, 1822, Truly Living in the Southwestern Atlantic

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*Crepidula fecunda* Gallardo, 1979, was described from Bahía Chinquiue (41°31'S–73°03'W) in the Chilean Pa-

cific. The distribution ranges from the type locality, 41°S, to 45°S off the Chilean coast. According to Gallardo (1979), *C. dilatata* Lamarck, 1822, can only be differentiated by its direct development and the presence of embryos consuming nurse eggs. Adult morphological features are identical. Therefore, earlier records referring to the presence of *C. dilatata* in the Atlantic coast of South America (Parodiz, 1939) need validation. The type locality of *C. dilatata* remains unknown. Mermod (1950) mentioned in a commented list of the types from Lamarck's collection, the Western coast of South America as a probable type locality. Gallardo (1979) recorded *C. dilatata* Lamarck, from 21°11'S to 43°47'S. He also stated (in Spanish in the original): "It is probable that future studies including developmental stages, will expand this distribution particularly towards the Argentine Atlantic coast."

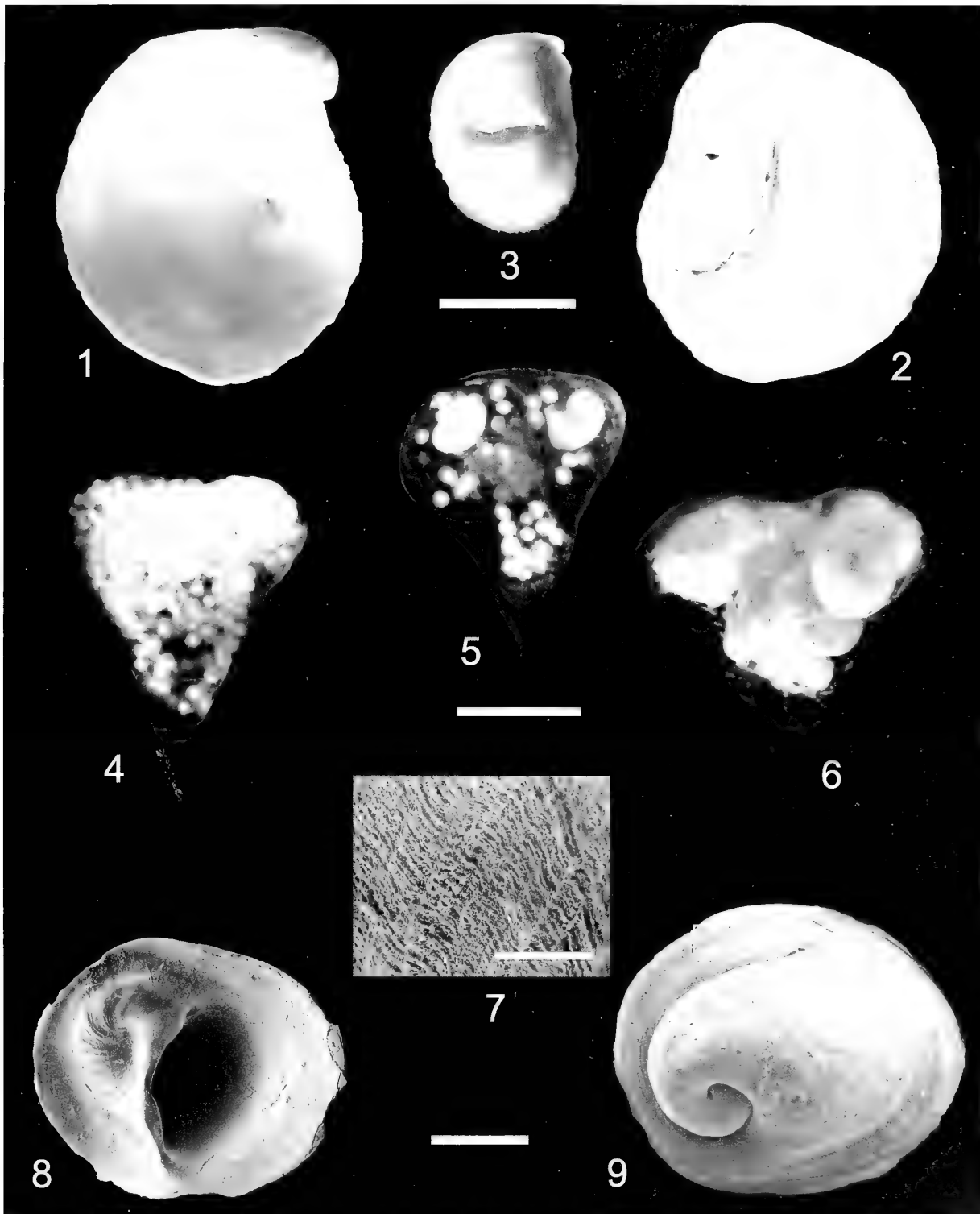
This note confirms the presence of *C. dilatata* (Figures 1–9) in Argentine waters and restricts *C. fecunda* to Chile.

Egg capsules and adult males and females were collected from Bahía Ensenada, Ushuaia (~55°S) by SCUBA diving in 3–4 m depth, attached to the root of the common kelp *Macrocystis pyrifera* (Linnaeus); Punta Peñas, Puerto San Julián (49°15'S–67°39'W) in 2 m depth; and Punta Dos Hermanas, Puerto Deseado (47°10'S; 2–3 m depth) in Santa Cruz province; and several localities around Golfo Nuevo (~42°30'S) in Chubut province (subtidal). All collections were made during February 2000.

We studied more than 100 brooding females (voucher material was deposited in Museo Argentino de Ciencias Naturales, number MACN 33901). Most females were brooding egg masses at advanced stages of embryonic development, containing embryos and uncleaved nurse eggs or crawling juveniles. This fact confirms the presence of *C. dilatata* in the southern Atlantic, and as far as we observed, restricts *C. fecunda* to the Pacific.

The observed material was completely homogeneous, with only one developmental mode characterized by the presence of nurse eggs. Each egg capsule ( $n = 150$ ) contained 203–375 eggs (mean = 303, SD = 54) with only two to 12 developing embryos, representing as an average 2.4% of the initial egg number. The average uncleaved egg diameter was 214  $\mu\text{m}$  (SD = 13  $\mu\text{m}$ ,  $n = 72$ ). The egg diameter distribution adjusted to a normal distribution with a single mode was 212  $\mu\text{m}$ . Hatching occurred at a crawling juvenile stage. The egg capsule size averaged 3873  $\mu\text{m}$  in length and 3954  $\mu\text{m}$  in width (SD = 648 and 527  $\mu\text{m}$ , respectively). Brooding females measured 11–32 mm (mean = 22 mm) in shell length, but in this protandric species the loss of penis can be already observed at 11 mm of shell length. Males (with a penis) measured 7–19 mm of shell length.

The Argentine material agrees with Gallardo's (1976,



Figures 1–9. *Crepidula dilatata*. Figure 1. Internal view of a female shell; scale bar = 1 cm. Figure 2. Dorsal view of the same specimen with a male shell in stacking position. Figure 3. Internal view of the male specimen from Figure 2. Figure 4. Egg capsule removed from a just laid egg mass; scale bar under 5. Figure 5. Egg capsule with embryos and remaining nurse eggs; scale bar = 2 mm. Figure 6. Egg capsule just prior to hatching with no remaining nurse eggs; scale bar under 5. Figure 7. SEM detail of the ornamentation of the embryonic shell in Figure 8; scale bar = 50  $\mu$ m. Figure 8. SEM, apertural view of the larval shell; scale bar = 0.5 mm. Figure 9. SEM, dorsal view of the embryonic shell; scale bar = 0.5 mm.

Table 1

Comparison of reproduction of Argentine and Chilean specimens of *Crepidula dilatata* Lamarck, 1822.

Source	Egg capsules per egg mass	Eggs per egg capsule	Embryos per egg capsule	Egg diameter ( $\mu\text{m}$ )	Crawling juvenile length ( $\mu\text{m}$ )	Male shell length ( $\mu\text{m}$ )	Female shell length ( $\mu\text{m}$ )
Gallardo, 1979	22–29	308–1016	15–18	195–263	900–1300	6–26	12–53
Chaparro & Paschke, 1990	—	—	—	—	1075–1600	—	—
Present study	9–22	203–375	2–12	197–263	740–1600	7–19.1	11–32

Table 2

Regression and correlation analysis of eggs and egg capsules of *Crepidula dilatata* Lamarck, 1822.

	$r^2$	F	m	P
Egg capsule length-number of eggs per egg capsule (n = 150)	0.33	7.25	0.05	0.05
Number of egg capsules per egg mass-egg capsule length (n = 30)	0.14	4.39	54.8	0.05
Number of egg capsules per egg mass-number of eggs per egg capsule (n = 30)	0.07	1.15	3.57	0.05
Egg capsule length-egg capsule width (n = 150)	0.24	8.63	0.4	0.05

1977, 1979) and Gallardo & Garrido's (1987) general description of the reproduction of *C. dilatata*. We noted, however, some differences. The maximum adult shell length was lower in the Argentine samples than those from Chile (Table 1). This fact could account for the fewer egg capsules per brood found in the Argentinean material (Table 1). There were also fewer developing embryos per egg capsule in the Atlantic sample compared with those studied by Gallardo (1979). The uncleaved egg diameter was between 197 and 263  $\mu\text{m}$ , similar for the Chilean population (Table 1), but we never found two different groups of egg diameters as suggested by Gallardo (1976, 1979). Further studies with a greater number of animals from both sides of the continent would clarify this matter.

There is a linear relationship between the number of egg capsules per brood and the number of eggs. When the egg mass has more egg capsules, each egg capsule is larger and contains more eggs (Table 2).

The number of eggs per capsule, the presence of nurse eggs, the hatching shell size, and the hatching stage as crawling juveniles agree with *C. dilatata*'s reproductive pattern as described by Gallardo (1979) and Chaparro & Paschke (1990). We therefore consider the presence of *C. dilatata* along the Argentine coast to be truly demonstrated. As far as we know, there is no evidence to include *C. fecunda* in the South Atlantic fauna.

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tive Argentina-Brazil-Chile Research Grant from Fundación Antorchas, Argentina and the project BID 802/OC-AR-PICT No. 01-04321 from the National Agency for Scientific and Technical Promotion, Argentina. Two anonymous reviewers kindly improved the manuscript.

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## BOOKS, PERIODICALS & PAMPHLETS

### **The Biology of Terrestrial Molluscs**

Edited by G. M. BARKER. 2002. CABI Publishing, Wallingford, UK. xiv + 558 pp. ISBN 0 85199 318 4. Price \$US 130.00.

With contributions by 22 authors, this volume offers a synthesis of current knowledge and research on land snail and slug biology that will be useful to specialists and general biologists alike. Chapter topics include phylogeny, diversity and adaptive morphology (G. M. Barker); body wall form and function (D. L. Luchtel and I. Deyrup-Olsen); nervous system and sensory organs (R. Chase); radular structure and function (U. Mackenstedt and K. Märkel); digestive system structure and function (V. K. Dimitriadis); food and feeding behavior (B. Speiser); haemolymph and blood cell function (E. Furuta and K. Yamaguchi); structure and functioning of the reproductive system (B. J. Gómez); regulation of growth and reproduction (A. Gomot de Vaufléury); spermatogenesis and oogenesis (J. M. Healy); population and conservation genetics (T. Backeljau, A. Baur, and B. Baur); life history strategies (J. Heller); behavioral ecology (A. Cook); and soil biology and ecotoxicology. All chapters contain extensive bibliographies; all were peer-reviewed. I found the text to be exceptionally readable (quite likely a testament to the skill of the editor as well as the authors). Illustrations are not superabundant but are adequate where called for. The result is an authoritative manual that should be a benchmark for terrestrial mollusk biology for years to come.

**B. Roth**

### **The Recent Molluscan Marine Fauna of Isla de Malpelo, Colombia**

by K. L. KAISER and C. W. BRYCE. 2001. The Festivus 33, Occasional Paper 1, 149 pp. Available from San Diego Shell Club, 3883 Mt. Blackburn Ave., San Diego, CA 92111 USA. \$US 30.00 within United States; \$35.00 Canada and Mexico; \$40.00 airmail to destinations outside the United States.

Here is another solid contribution from the publication program of the San Diego Shell Club, a spiral-bound, annotated faunal list, thoroughly illustrated with 49 black-and-white plates and five color plates of living mollusks in their natural habitats. The new information contained is largely based on two expeditions by the authors, in 1998 and 2000, during which they systematically sampled the intertidal and subtidal shallow-water habitats of Isla de Malpelo. Their painstaking work increased the known number of mollusk species from Isla de Malpelo and its surrounding waters from 83 to 341. The numerous illustrations are of uneven but mostly very good quality, including crisp SEM images of the most minute items. Many small specimens were identifiable only to genus (and in some cases only to family); these are signaled in the list and illustrations as "sp. 1," "sp. 2" and so forth. The authors have resisted any temptation to describe new taxa; rather, this work will be an invaluable starting point for further study to clarify the identities of these mysterious customers, and hence the biogeography of the island malacofauna as a whole.





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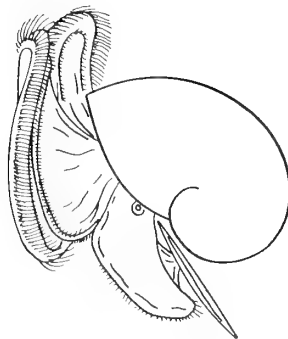
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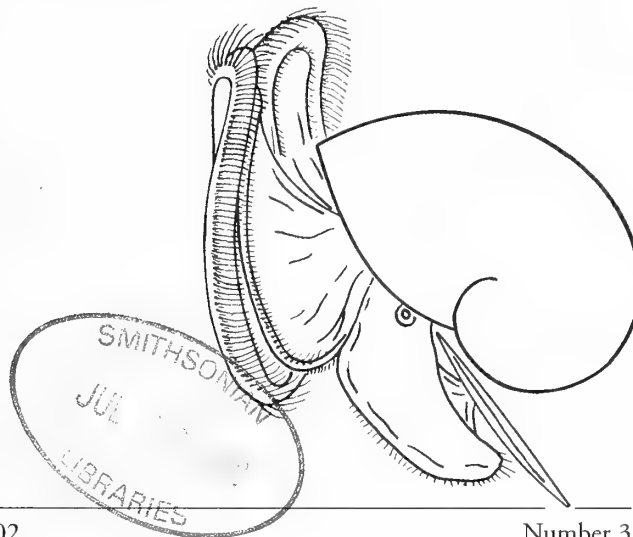


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## New Information on Late Cretaceous, Paleocene, and Eocene Neritid Gastropods from the North American Pacific Slope

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**Abstract.** Nine species of neritid gastropods from shallow-marine, Upper Cretaceous, Paleocene, and Eocene rocks of the Pacific slope of North America are discussed. Two are new species: *Nerita* (*Bajanerita*?) **banosensis**, sp. nov., from the Upper Cretaceous (Maastrichtian Stage) “Quinto Silt” member of the Moreno Formation, Merced County, north-central California; and *Nerita* (*Theliostyla*?) **kennedyi**, sp. nov. from the upper lower to lower middle Eocene (“Domengine Stage”) Santiago Formation, near Vista, northern San Diego County, southern California.

An immature specimen of *Corsania* (subgenus?) sp., from unnamed lower Upper Cretaceous (Cenomanian Stage) strata near Dayville, Grant County, east-central Oregon, represents the first confirmed Cenomanian record of a neritid from the Pacific slope of North America.

*Corsania* (*Januncia*) *rhoga* Saul & Squires, 1997, previously known only from lower Paleocene strata in Lake County, northern California, is reported from lower? and upper Paleocene strata in Los Angeles County, southern California. The late Paleocene *Corsania* (*Januncia*) *janus* Woods & Saul, 1986, previously known only from Baja California Sur, Mexico, is reported from Santa Cruz Island, southern California.

*Nerita* (*Theliostyla*) *triangulata* Gabb, 1869, a widespread Eocene species is reported for the first time from Washington. Previously unknown, early juvenile morphology and color patterns are described for this species. *Nerita washingtoniana* Weaver & Palmer, 1922, is synonymized with *N. (T.) triangulata*, and *Nerita cowlitzensis* Dickerson, 1915, is questionably synonymized with the latter. *Neritina martini* Dickerson, 1915, an Eocene species from Washington is tentatively assigned to subgenus *Neritina*, previously known only from the modern record.

### INTRODUCTION

Recent field and museum work resulted in the discovery of rare specimens of Late Cretaceous and Early Cenozoic neritid gastropods from the Pacific slope of North America. Two new species, a possible new species, and new information about six other previously known species of neritids were the results of this study. The general areas of the type localities of the new species, as well as the new geographic occurrences of these other species, are shown in Figure 1.

Neritid gastropods are relatively uncommon in the rock record of the northeastern Pacific. This scarcity is due to a variety of reasons. These gastropods commonly lived in rocky shoreline habitats, and these are normally not preserved in the rock record. Also, the record is not continuous because marine neritids, which are warm-water gastropods, only lived in this area during periods of warm climate. In addition, many fossil neritids are overlooked because they resemble naticid gastropods (Saul & Squires, 1997).

The sequence of North American Pacific slope Paleocene and Eocene molluscan stages used in this report was recently put into the current chronostratigraphic framework by Squires (in press). These stages are the following: “unnamed stage” (early Paleocene); “Martinez Stage” (late Paleocene); “Meganos Stage” (latest Paleocene to earliest Eocene); “Capay Stage” (middle early Eocene); “Domengine Stage” (late early to early middle Eocene); “Transition Stage” (early middle Eocene); “Telson Stage” (middle to late middle Eocene); and Galvinian Stage (late Eocene to earliest Oligocene). These stages, along with the Upper Cretaceous ones, are shown in Figure 2.

Abbreviations used are: CAS, California Academy of Sciences, San Francisco; LACM, Natural History Museum of Los Angeles County, Malacology Section; LAC-MIP, Natural History Museum of Los Angeles County, Invertebrate Paleontology Section; SDSNH, San Diego Museum of Natural History, San Diego; UCLA, University of California, Los Angeles (collections now stored at



Figure 1. Index map to type localities of the new species and new geographic occurrences of previously named neritids discussed in this study. 1 = "Big Bend" of Cowlitz River near Vader. 2 = Near Dayville. 3 = Los Banos Creek. 4 = Trailer Canyon. 5 = Santa Cruz Island. 6 = Near Vista.

LACMIP); UCMP, University of California Museum of Paleontology (Berkeley); UCR, University of California, Riverside; UWBM, University of Washington (Seattle), Thomas Burke Memorial Washington State Museum [= UW in older literature].

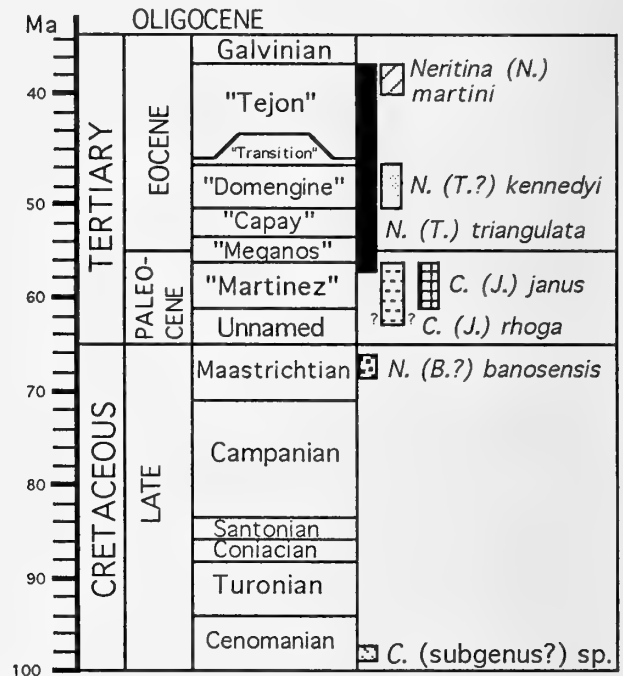


Figure 2. Age and stratigraphic positions of the neritids discussed in this study. Cretaceous stages time scale from Gradstein et al. (1994); Tertiary stages time scale from Squires (in press). *Nerita (Theliostyla) triangulata* range includes the synonym *Nerita washingtoniana* and the questionable synonym *Nerita cowlitensis*.

## SYSTEMATIC PALEONTOLOGY

Family NERITIDAE Rafinesque, 1815

Subfamily NERITINAE Rafinesque, 1815

Genus *Corsania* Vidal, 1917

**Type species:** *Corsania douvillei* Vidal, 1917, by original designation; late Early Cretaceous (Aptian Stage), Cors, Lérída, Spain.

*Corsania* (subgenus?) sp.

(Figures 3–5)

**Description:** Shell minute (5.4 mm high), broader than high, consisting of about nearly two whorls, spire lowly elevated, body whorl rapidly expanding; suture impressed. Shoulder of body whorl angulate with broad, low-sloping to very slightly concave ramp. Growth lines on ramp prosocline. Body whorl smooth, convex. Aperture moderately large. Deck area narrow. Inner lip slightly irregular with several, very minute prominences (teeth?), especially posteriorly. Outer lip smooth.

**Material examined:** Hypotype LACMIP 12905 from LACMIP loc. 9936.

**Distribution:** Unnamed strata about 9.5 km southeast of



Dayville, Grant County, east-central Oregon (LACMIP loc. 9936).

**Geologic age:** Late Cretaceous (early Cenomanian Stage).

**Discussion:** The only known specimen of this species is, most likely, an early juvenile, based on its minute size. It is probably a new species, but it is not named at this time because of the incompleteness of knowledge about its morphology as an adult. To name it would only cause problems for future workers in their attempts to make morphologic comparisons.

Squires & Saul (2002) reported an early Cenomanian age and shallow-marine paleoenvironment for the rocks found at LACMIP loc. 9936 near Dayville. They also reported new species of iteriid and actaeonellid gastropods from the same locality.

The Oregon specimen has a high, wide shoulder and rapidly enlarging body whorl which are like those found in *Corsania*. This genus is characterized by ornament consisting of spiral ridges with tubercles crossed by col-labral ridges (on portions of the whorls), as well as by teeth on the inner lip (Woods & Saul, 1986). The Oregon specimen does not have any ornament, but this might be the result of having been worn by post-mortem transport, or it could be related to an early juvenile-growth stage of the specimen. The specimen has some very minute irregularities on what appears to be the inner lip. It is possible, however, that the inner lip teeth have been resorbed, which is a common phenomenon in neritids (Woodward, 1892; Cossmann, 1925). Also, it is possible that the deck area, which is a callused area that encompasses the inner lip, has been detached. Broken deck areas are not uncommon in neritids, and Squires & Saul (1993) reported a fossil specimen whose deck area had been pushed into the aperture. The specimen from LACMIP loc. 9936 cannot be assigned to a subgenus because of the possibility that the deck area has been detached. There are two recognized subgenera of *Corsania*; namely, *Corsania* (*Corsania*) Vidal, 1917, and *Corsania* (*Januncia*) Woods & Saul, 1986. *Corsania* (*Januncia*) differs from the former by having a strongly depressed (sunken) deck area. The inner edge of this depressed deck area has a nearly straight trend behind the embellishment of the strong teeth, thereby imparting a double inner lip structure. Better preserved and more mature specimens of the Oregon species are needed in order to determine the subgenus of this species.

This *Corsania* (subgenus?) sp. is the first Cenomanian record of *Corsania* from the Pacific coast of North America. *Corsania* (*Corsania*) *allisoni* Saul & Squires (1997: 139, 141, figs. 22–24) from the Lower Cretaceous (middle Albian) upper member of the Alisitos Formation, Baja California, Mexico, is the earliest *Corsania* on the Pacific coast of North America and the only other record of this genus in this region. *Corsania* (*Corsania*) probably orig-

inated in the Old World Tethyan paleobiologic province during the Lower Cretaceous Aptian Stage (Saul & Squires, 1997). *Corsania* (subgenus?) sp. differs from *C. (C.) allisoni* by not having any ornament, but, as mentioned above, this might be the result of poor preservation and/or growth stage.

The only other neritid on the Pacific coast of North America that might range into the Cenomanian is the Cretaceous *Otostoma? atopus* Saul & Squires (1997:138–139, figs. 19–21) known from reworked clasts of late Albian-early Cenomanian age in the Venado Formation of Late Cretaceous (early Turonian) age, northern California. Because of the uncertainty as to exact geologic age of *O. ? atopus*, *Corsania* (subgenus?) sp. represents the first confirmed record of a neritid from the Cenomanian Stage of the Pacific slope of North America and extends the northern range of Albian-Cenomanian neritids in this region. *Corsania* (subgenus?) sp. differs from *O. ? atopus* by having a much lower spire, a low-sloping to slightly concave ramp, and no prominent inner lip teeth.

#### Subgenus *Januncia* Woods & Saul, 1986

**Type species:** *Corsania* (*Januncia*) *janus* Woods & Saul, 1986, by original designation; late Paleocene (“Martinez Stage”), Baja California, Mexico.

#### *Corsania* (*Januncia*) *rhoga* Saul & Squires, 1997

(Figures 6–8)

*Corsania* (*Januncia*) *rhoga* Saul & Squires, 1997:142, figs. 25–27.

**Holotype:** LACMIP 7889.

**Type locality:** LACMIP loc. 7047, unnamed rocks, Lake County, northern California.

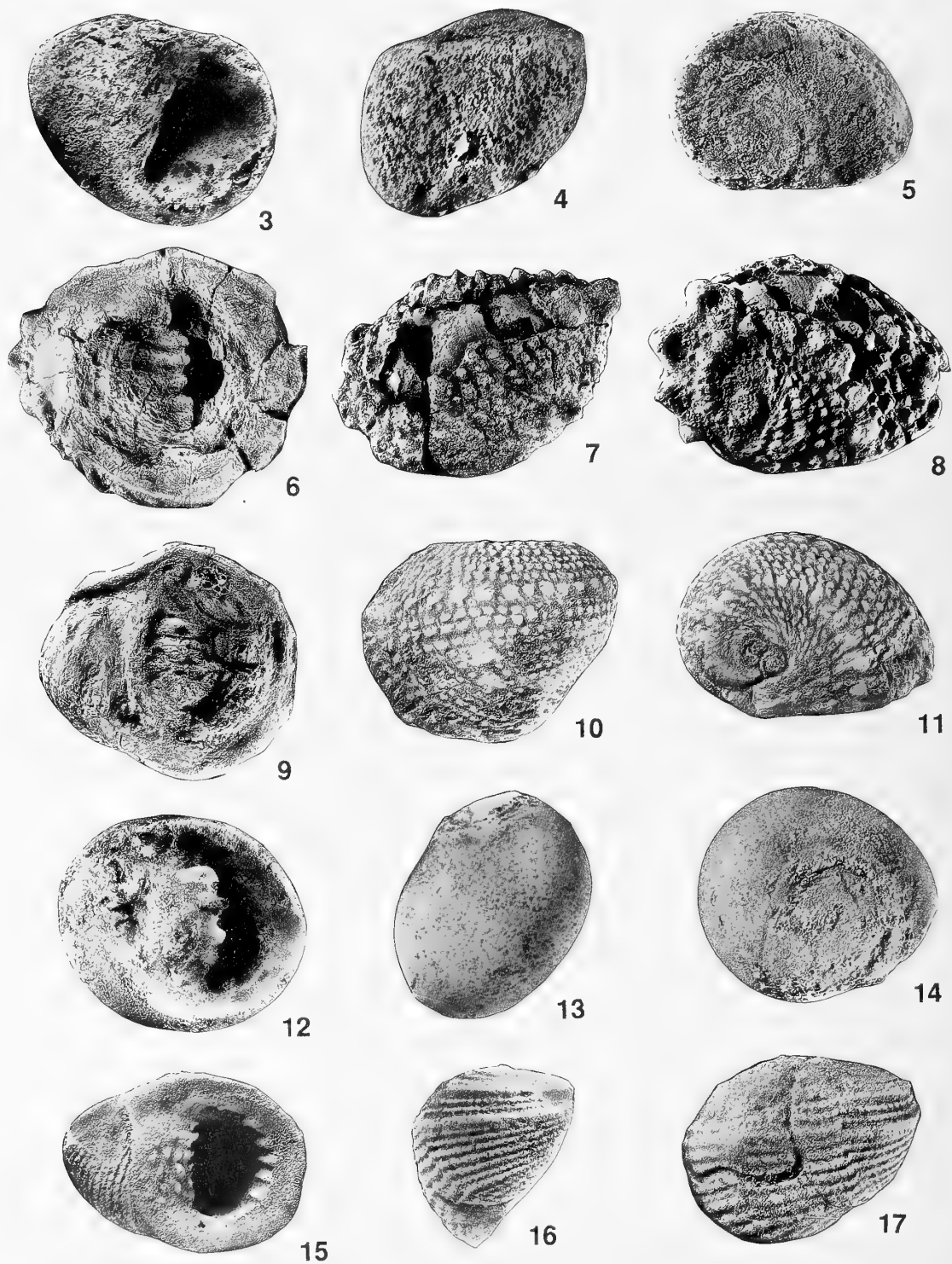
**Other material examined:** Hypotype LACMIP 12906 from LACMIP loc. 10508, and a specimen from LACMIP loc. 26720.

**Distribution:** Upper part of Santa Susana Formation, Trailer Canyon, Santa Monica Mountains, southern California (LACMIP locs. 10508 and 26720) and unnamed rocks, Lake County, northern California (LACMIP loc. 7047).

**Geologic age:** Late early? Paleocene (late “unnamed stage”?) to late Paleocene (“Martinez Stage”).

**Discussion:** Two specimens were found. One is from LACMIP loc. 10508 and is the largest (36.7 mm high and 53 mm wide) and most complete specimen of *C. (J.) rhoga* (Figures 6–8). This specimen shows, for the first time, the entire inner lip. Five teeth are present, and the two posteriormost ones are the most developed. The other specimen, which is from LACMIP loc. 26720, is complete but does not show the inner lip very well.

The new specimens of *C. (J.) rhoga* from the Santa



Figures 3–17. All specimens coated with ammonium chloride. Figures 3–5. *Corsania* (subgenus?) sp., hypotype LACMIP 12905, LACMIP loc. 9936, Dayville area, Oregon, height 5.4 mm,  $\times 5.7$ . Figure 3. Apertural view. Figure 4. Abapertural view. Figure 5. Apical view. Figures 6–8. *Corsania* (*Januncia*) *rhoga* Saul & Squires, 1997, hypotype LACMIP 12906, LACMIP loc. 10508, Santa Monica

Monica Mountains are only the second and third known specimens of this species. They significantly extend the geographical range of *C. (J.) rhoga* southward by 650 km and extend the geologic range upward into the late Paleocene. At the new locality (LACMIP loc. 10508), *C. (J.) rhoga* was found in a coralline-algal-rich, micaceous muddy siltstone about 1 m stratigraphically below a 24-m-thick blocky, coralline-algal-limestone interval. The specimens of *C. (J.) rhoga* were found among numerous specimens of the gastropod *Mesalia clarki* (Dickerson, 1914) and articulated specimens of the bivalves *Plicatula lapidicina* Squires & Saul, 1998, and *Plicatula trailerensis* Squires & Saul, 1998. The rocks that compose LACMIP loc. 10508 were interpreted to be of late Paleocene age and deposited very nearshore, under tropical to subtropical conditions (Squires, 1993a; Squires & Kennedy, 1998; Squires & Saul, 1998).

*Januncia* originated in the Old World Tethyan paleobiotic province, and the earliest known species is known from the Maastrichtian or Danian of western Iran (Woods & Saul, 1986). *Corsania (J.) rhoga* is the earliest known species of this subgenus on the Pacific slope of North America.

*Corsania (Januncia) janus* Woods & Saul, 1986

(Figures 9–11)

*Corsania (Januncia) janus* Woods & Saul, 1986:640–641, figs. 5.1–5.6.

**Type specimens:** Holotype UCLA 59426; paratypes UCLA 59427–59430.

**Type locality:** LACMIP loc. 27083, Sepultura Formation, east of Bahía Sebastian Vizcaino, Baja California, Mexico.

**Other material examined:** Hypotype LACMIP 12907 from LACMIP loc. 23348.

**Distribution:** Sepultura Formation, east of Bahía Sebastian Vizcaino, Baja California, Mexico (LACMIP loc. 27083) and Pozo Formation, Well Canyon, Santa Cruz Island, southern California (LACMIP loc. 23348).

**Geologic age:** Late Paleocene (“Martinez Stage”).

**Discussion:** A single specimen is known from the Pozo Formation on Santa Cruz Island. This specimen (Figures

9–11), which is the largest known for this species, is 30.5 mm high and 39 mm wide. The specimen is well preserved exteriorly, but interiorly the deck area is very thin and impossible to clean entirely without destroying it. Careful partial cleaning, however, revealed that the deck area is strongly depressed, which is a diagnostic feature of *Januncia*. The cleaning also revealed three of the six elongate inner lip teeth that characterize *Corsania (Januncia) janus*.

Woods & Saul (1986) mentioned that *C. (J.) janus* is similar to *C. (J.) limata* (White, 1887:196, pl. 15, figs. 6, 7) from Paleocene rocks in Brazil, and the Pozo Formation specimen confirms this comparison.

Doerner (1969) mentioned the same bed (i.e., LACMIP loc. 23348) that yielded the hypotype (LACMIP 12907) of *C. (J.) janus*. He reported that the molluscan fauna in this bed had lived in shallow, inshore waters of a semi-tropical to tropical environment. Using the presence of *Turritella pachecoensis* Stanton, 1896, he assigned a Paleocene age to the fauna. Saul (1983) considered *T. pachecoensis* to be a subspecies; namely, *Turritella infragranulata pachecoensis* Stanton, 1896. Saul (1983) assigned the rocks from LACMIP loc. 23348 to the “Martinez Stage” of late Paleocene age.

The Pozo Formation specimen of *C. (J.) janus* provides data on the minimum size of this species’ range, which is relatively large. Previously, this species was known only from the Punta Rosarito area, northern Bahía Sebastian Vizcaino, on the western coast of Baja California, Mexico. Today, Santa Cruz Island is about 650 km north of Punta Rosarito. During the Eocene, however, the Pozo Formation was situated 150 km farther south and near what is now known as San Diego. During the Late Cenozoic, Santa Cruz Island underwent about 150 km of clockwise tectonic rotation to its present-day position (Atwater, 1998), and when this rotation is removed, the Pozo Formation occurrence of *C. (J.) janus* actually represents only a 500-km-range extension to the north.

Genus *Nerita* Linnaeus, 1758

**Type species:** *Nerita peloronta* Linnaeus, 1758, by subsequent designation (Montfort, 1810); Recent, South Florida, West Indies, and Bermuda.

Mountains, California, height 36.7 mm,  $\times 0.9$ . Figure 6. Apertural view. Figure 7. Abapertural view. Figure 8. Apical view. Figures 9–11. *Corsania (Januncia) janus* Woods & Saul, 1986, hypotype LACMIP 12907, LACMIP loc. 26720, Santa Cruz Island, California, height 30.5 mm,  $\times 1.1$ . Figure 9. Apertural view. Figure 10. Abapertural view. Figure 11. Apical view. Figures 12–14. *Nerita (Bajanerita?) banosensis* Squires & Saul, sp. nov., holotype LACMIP 12908, LACMIP loc. 10676, Los Banos Creek, California, height 9 mm,  $\times 4.1$ . Figure 12. Apertural view. Figure 13. Abapertural view. Figure 14. Apical view. Figures 15–17. *Nerita (Theliosyla) triangulata* Gabb, 1869, hypotype LACMIP 12909, LACMIP loc. 6298, “Big Bend” of Cowlitz River, Washington, height 7.5 mm,  $\times 4.1$ . Figure 15. Apertural view. Figure 16. Lateral view. Figure 17. Abapertural view.

Subgenus *Bajanerita* Squires, 1993

**Type species:** *Nerita (Bajanerita) californiensis* (White, 1885), by original designation; Late Cretaceous, Baja California, Mexico.

**Discussion:** *Bajanerita* has an inner lip with a convex trend, and this is one of the main distinguishing features of this subgenus. Re-examination of many specimens of the type species of *Bajanerita* revealed that this genus is also characterized by the presence of a subsutural collar anterior to the suture. Strength of this collar is variable. In addition, the growth lines change from prosocline to nearly straight as they pass from the shoulder onto the collar area. This subsutural collar and its variability in strength are evident in photographs provided by Squires (1993b:figs. 2.3, 2.4, 2.6, 2.8).

*Nerita (Bajanerita?) banosensis* Squires & Saul,  
sp. nov.

(Figures 12–14)

**Diagnosis:** Smooth shell, barely elevated spire, inner lip with four squarish teeth, and a moderately swollen callus.

**Description:** Shell small (9 mm high), naticiform/neritiform, convex, thin-shelled, consisting of approximately 2½ whorls; spire barely elevated, body whorl rapidly expanding, early whorls nearly hidden by body whorl; suture impressed. Subsutural collar anterior to suture very faint. Body whorl smooth. Growth lines prosocline. Aperture moderately large, subcircular; apertural opening moderately narrow. Deck callus moderately swollen, smooth. Trend of inner lip convex; inner lip with four teeth, squarish, equidistant; posteriormost tooth strongest. Outer lip smooth.

**Dimensions of holotype:** Height 9 mm, width 8 mm.

**Holotype:** LACMIP 12908.

**Type locality:** LACMIP loc. 10676, 36°59'28"N, 120°55'50"W, Moreno Formation, informal "Quinto Silt" member (see Anderson, 1958), Los Banos Creek, Merced County, north-central California.

**Other material examined:** A specimen from LACMIP loc. 10676, and a specimen from LACMIP loc. 10685.

**Distribution:** "Quinto Silt" member of Moreno Formation, Los Banos Creek, Merced County, north-central California (LACMIP locs. 10676 and 10685).

**Geologic age:** Late Cretaceous (middle Maastrichtian Stage).

**Discussion:** Three specimens were found. Two are from LACMIP loc. 10676, and of these, one is complete and the other is a fragment. The specimen from LACMIP loc. 10685 is also a fragment. Both localities are in close proximity to each other in Los Banos Creek. The spire

on the holotype is slightly crushed, and the growth lines on the body whorl are poorly preserved, especially in the vicinity of the suture. None of the specimens shows any teeth on the outer lip, but this might just be a function of growth.

The new species has a convex inner lip, a very faint subsutural collar, and the additional following features of *Bajanerita*: smooth body whorl and several squarish teeth on the inner lip. The new species, however, has four teeth on the inner lip, whereas *Bajanerita* has only three. The new species might belong to *Bajanerita* or to a closely allied subgenus.

*Bajanerita* is known only from the Pacific slope of North America. Its earliest record is *Nerita (Bajanerita) californiensis* (White, 1885), from the Upper Cretaceous (upper Campanian to lower Maastrichtian stages) Rosario Formation at Punta Banda, Baja California, Mexico, and Jalama Formation, Santa Barbara County, southern California (Saul & Squires, 1997). Ascending biostratigraphically, two additional possible species of *Bajanerita* are the following: "Capay Stage" *Nerita (Bajanerita?) larix* Saul & Squires, 1997, from the upper part of the Crescent Formation, southwestern Washington; and Galvinian Stage *Nerita (Bajanerita?) vokesi* Durham, 1944, from southwestern Washington (Saul & Squires, 1997).

The new species differs from *Nerita (Bajanerita) californiensis* (White, 1885:pl. 5, figs. 7, 8; Squires, 1993b, figs. 2.1–2.8) by having a much lower spire, a much weaker subsutural collar, four rather than three inner lip teeth, a wider callus, and no outer lip teeth. The new species differs from *Nerita (Bajanerita?) larix* Saul & Squires (1997:136–137, figs. 9–11) by having a much lower spire, wider inner lip teeth, and no outer lip teeth. The new species differs from *Nerita (Bajanerita?) vokesi* Durham (1944:156, pl. 17, figs. 11, 12) by having an inner lip with a convex rather than a straight trend and a larger shell size. There might be other differences, but as Saul & Squires (1997) pointed out, the morphology of *N. (B.?) vokesi* is poorly known.

At both localities in Los Banos Creek where the new species was found, the bivalve *Glycymeris banosensis* Anderson, 1958, is very abundant. Saul (1983) referred to this bivalve as *Glycymeris (Glycymerita?) banosensis* and interpreted that the specimens are in situ and that they lived in a shallow-water environment. Also present at LACMIP loc. 10685 is the bivalve *Calva (Calva) varians* (Gabb, 1864) of middle to late Maastrichtian age (Saul & Popenoe, 1992), the gastropod *Gyrodes (Sohlella) expansus* Gabb, 1864, of middle to late Maastrichtian age (Popenoe et al., 1987), and the gastropod *Perissititys stantoni* (Stewart, 1927) of late Maastrichtian age (Popenoe & Saul, 1987). Based on association with these last-mentioned three species, the new species is assigned a middle Maastrichtian age, near the middle-late Maastrichtian boundary.

**Etymology:** The species is named for Los Banos Creek, California where the type locality of the new species is located.

### Subgenus *Theliostyla* Mörch, 1852

**Type species:** *Nerita albicilla* Linnaeus, 1758, by subsequent designation (Kobelt, 1879); Recent, Indo-Pacific.

### *Nerita (Theliostyla) triangulata*

(Figures 15–27)

*Nerita (Theliostyla) triangulata* Gabb, 1869:170, pl. 28, figs. 52, 52a; Vokes, 1939:182, pl. 22, figs. 31, 33, 34; Givens, 1974:61, pl. 5, fig. 4; Givens & Kennedy, 1976:960, 963, pl. 1, figs. 1–4; Devjatilova & Volobueva, 1981:108, pl. 9, figs. 2–4; Squires, 1987:23, fig. 14; 1992:325–327, figs. 2–18; 1994:48, pl. 2, fig. 6; Oleinik, 1998:383–384, pl. 3, figs. 1, 2.

*Nerita triangulata* Gabb: Arnold, 1910:14, pl. 14, figs. 12, 12a (figs. repeated in Arnold & Anderson, 1910:pl. 26, figs. 12, 12a); Hanna, 1927:301, pl. 46, figs. 11, 12, 16, 17; Moore, 1968:28, pl. 12a.

? *Nerita cowlitzensis* Dickerson, 1915:58–59, pl. 5, figs. 7a, b; Weaver, 1943:294–295, pl. 63, fig. 11; Nesbitt, 1995: table 1.

*Nerita washingtoniana* Weaver & Palmer, 1922:28–29, pl. 11, fig. 4; Weaver, 1943:295, pl. 64, fig. 8.

*Nerita triangulata* Gabb var. *oregonensis* Merriam & Turner, 1937:104, pl. 6, fig. 5; Turner, 1938:95, pl. 19, figs. 10–12; Weaver, 1943:295–296, pl. 64, figs. 10, 13.

*Nerita* n. sp.: Clark, 1938:701, pl. 4, fig. 6.

*Nerita quadrangulata* Weaver & Klempell, 1963:183, pl. 23, fig. 1.

**Description of juveniles:** Shell minute (2 to 5 mm high), broader than high, with rapidly expanding body whorl. Spire very low to flattened, apex usually depressed. Posterior part of dorsal surface elevated. Dorsal surface with extremely faint and noded spiral ribs or with distinct, noded spiral ribs. Body whorl with carinate shoulder and, usually, another carina a short distance anteriorly. Very closely spaced, unnoded spiral ribs cover most of body whorl, except near base of whorl. Anteriormost spiral rib carinalike toward outer lip. Aperture large, quadrate (rarely elliptical). Outer lip flared with seven to 10 teeth, not extending to outer lip periphery. Two posteriormost teeth stronger than rest, with tooth next to posteriormost tooth strongest. Three to four small, subequal teeth medially. Deck with five to six granules, arranged loosely in rows. Color bands axial, wavy or non-wavy; some non-wavy bands bifurcate and others do not extend to shell apex. Growth lines prosocline.

**Holotypes:** Of *N. (T.) triangulata*, type material missing (*vide* Keen & Bentson, 1944:179). Of *N. cowlitzensis*. CAS 183.02 [= CAS 290]; of *N. washingtoniana* CAS 66548.01 [= UW 197 = CAS 7591].

**Type localities:** Of *N. (T.) triangulata*, (exact location unknown), Domengine Formation, New Idria area, San

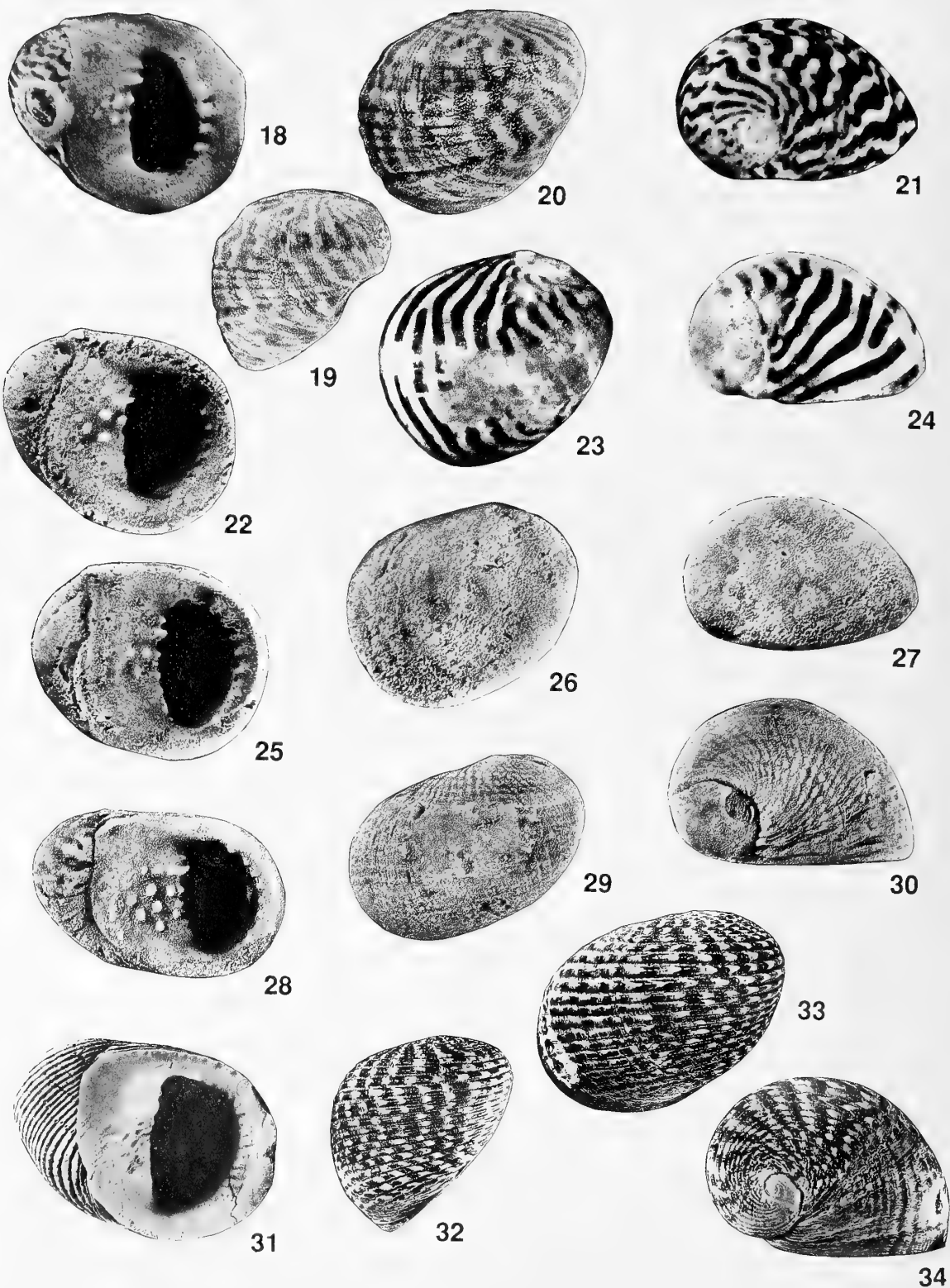
Benito County, central California. Of *N. cowlitzensis*, UWBM loc. 232, Cowlitz Formation, Lewis County, southwestern Washington. Of *N. washingtoniana*, UWBM loc. 329, Cowlitz Formation, Lewis County, southwestern Washington.

**Other material examined:** Hypotypes LACMIP 12909 to 12911 and seven other specimens from “Big Bend” of Cowlitz River, east of Vader, Lewis County, southwestern Washington.

**Distribution:** San Diego, southern California to southwestern Washington; also present in northwestern Kamchatka.

**Geologic age:** Late Paleocene (“Martinez Stage”) through late middle Eocene (“Tejon Stage”). “MARTINERENTIATED: Kamchikskaya Formation and Tkapravayamskaya Formation, Cape Getkilnin, northwestern Kamchatka (Oleinik, 1998); “CAPAY STAGE”: Capay Formation, Yolo County, northern California and White Tail Ridge formation (informal) [formerly referred to as the upper Umpqua formation (informal) and the Lookingglass Formation (see Squires, 1998)], southwestern Oregon (Merriam & Turner, 1937; Turner, 1938); “DOMENGINE STAGE”: Delmar Formation, San Diego, southern California (Hanna, 1927; Givens & Kennedy, 1979; “Santiago Formation” (formerly referred to as the Delmar Formation, in the Vista area, northern San Diego County (Givens & Kennedy, 1976; Squires, 1992); Matilija Sandstone, Pine Mountain area, Ventura County, southern California (Givens, 1974); Matilija Sandstone?, Whitaker Peak area, Ventura County, southern California (Squires, 1987); Domengine Formation, Coalinga area, central California (Vokes, 1939). “TEJON STAGE”: Sacate Formation-“Coldwater” Sandstone [formerly referred to as the undifferentiated Sacate-Gaviota Formation, Santa Barbara County, southern California (see Squires, in press)] (Weaver & Klempell, 1963); Markley Formation, Solano County, northern California (Clark, 1938); Cowlitz Formation, Lewis County, southwestern Washington (new information).

**Discussion:** Even though the shallow-marine to brackish-marine *Nerita (Theliostyla) triangulata* is the most geographically widespread (and the most geologically long-ranging) neritid gastropod in the Paleogene rock record of the northern Pacific, it is reported here for the first time from Washington. The Washington specimens (a total of 10) are all from the upper middle Eocene Cowlitz Formation (“Tejon Stage”) in the vicinity of the “Big Bend” of the Cowlitz River, east of Vader, Lewis County. The Cowlitz Formation was deposited at an estimated paleolatitude of 40°N to 45°N, in warm-tropical to subtropical, shallow-marine and marginal-marine environments associated with a prograding river-dominated delta (Nesbitt, 1995). This formation is chronostratigraphically near





the top of the "Tejon Stage" and ranges in age from approximately 39 to 36 Ma (Nesbitt, 1995; Squires, in press). The occurrence of *N. (T.) triangulata* in "Tejon Stage" rocks in Washington reinforces how far north warm-water mollusks ranged during the middle to late Eocene on the Pacific slope of North America.

*Nerita (T.) triangulata* is a rare taxon in the Cowlitz Formation (E. Nesbitt, personal communication). The largest known specimen (7.5 mm high) of this species from this formation is illustrated in Figures 15–17. This specimen shows all the diagnostic morphologic features described by Squires (1992) for *Nerita (Theliostyla) triangulata*. All the other known specimens of *N. (T.) triangulata* from the Cowlitz Formation are juveniles, and most of these are between 2 and 3 mm high. A few show color patterns. Many of the juvenile specimens from this formation were collected in bulk samples taken from LACMIP loc. 6297 by R. L. Squires. The morphology of the juvenile stage of *N. (T.) triangulata* was previously not known.

The holotype of *Nerita washingtoniana* Weaver & Palmer, 1922, is a worn juvenile 2 mm high (Figures 25–27). Unfortunately, the shell on the body whorl is missing. The overall shape of the shell and the morphological details of the aperture are identical to that of *Nerita (Theliostyla) triangulata*, although the granules on the deck area are less prominent because of abrasion. *Nerita washingtoniana*, therefore, is synonymized with *N. (T.) triangulata*. Weaver & Palmer (1922:295) mentioned that the color bands on their species "extend regularly over the surface of the body whorl without a zigzag or wavy pattern." They did not illustrate a specimen showing this original coloration, nor did they give a catalog number to any specimen that shows it. The specimen illustrated in Figures 22–24 fits their description, and this specimen was collected by R. L. Squires.

The holotype of *Nerita cowlitzensis* Dickerson, 1915, is a juvenile 5 mm high (Figures 28–30). It is a somewhat worn specimen, and the early part of the body whorl is missing its shell. Although the carina on the body whorl shoulder is evident, other carinae are poorly evident, and this is probably because of abrasion. A second carina, a

short distance anterior to the body whorl shoulder, is very faint.

Dickerson (1915) reported that the shoulder of *Nerita cowlitzensis* is less angulated than *N. (T.) triangulata*. Squires (1992) reported that *N. cowlitzensis* differs from *N. (T.) triangulata* by being smaller, nodose only on the dorsal surface, body whorl with only minute sculpture, and aperture more elongate. The apparent differences of angulation and sculpture could be explained by taking into account that the holotype of *N. cowlitzensis* is a worn specimen of an early juvenile. The aperture of the holotype of *N. cowlitzensis* is more elongate than is common in specimens of *N. (T.) triangulata*. This greater elongation might be the result of slight distortion during post-burial compaction, or it might be the result of a paleoenvironmental factor. Better preserved specimens of *N. cowlitzensis*, however, are needed to positively confirm whether or not these species are the same. We questionably synonymize them because, other than the apparent differences mentioned above, their deck areas, inner lips, and outer lips are identical.

*Nerita (Theliostyla?) kennedyi* Squires & Saul,  
sp.nov.

(Figures 31–34)

**Diagnosis:** A globose *Theliostyla* with a flattened spire, rounded body whorl, convex ramp, numerous subequal spiral ribs, low wrinkles and elongate nodes on deck callus, and a color pattern consisting of alternating collabral bands of light and dark.

**Description:** Shell medium small, broader than high, globose, 2¾ whorls, with rapidly expanding body whorl. Uppermost spire flattened, apex immersed. Suture impressed. Ramp convex. Body whorl shoulder rounded. Earliest 1½ whorls smooth, rest of teleoconch covered with numerous narrow, closely spaced spiral ribs with interspaces narrower than ribs; three to four spiral ribs on rounded body whorl shoulder slightly stronger and more widely spaced than elsewhere; spiral ribs on medial part of body whorl can be somewhat narrower with narrower

Figures 18–34. Specimens coated with ammonium chloride, unless otherwise noted. Figures 18–27. *Nerita (Theliostyla) triangulata* Gabb, 1869, Vader area, Washington. Figures 18–21. Hypotype LACMIP 12910, LACMIP loc. 22536, height 3 mm. Figure 18. Apertural view (uncoated), ×11. Figure 19. Lateral view, ×10. Figure 20. Abapertural view, ×11. Figure 21. Apical view (uncoated), ×10. Figures 22–24. Hypotype LACMIP 12911, LACMIP loc. 6297, height 2 mm, ×16.5. Figure 22. Apertural view. Figure 23. Abapertural view (uncoated). Figure 24. Apical view (uncoated). Figures 25–27. Holotype CAS 66548.01 of *Nerita washingtoniana* Weaver & Palmer, 1922, height 2 mm, ×16.5. Figure 25. Apertural view. Figure 26. Abapertural view. Figure 27. Apical view. Figures 28–30. ? *Nerita (Theliostyla) triangulata* Gabb, 1869, holotype CAS 183.02 of *Nerita cowlitzensis* Dickerson, 1915, height 5 mm, ×5.4. Figure 28. Apertural view. Figure 29. Abapertural view. Figure 30. Apical view. Figures 31–34. *Nerita (Theliostyla?) kennedyi* Squires & Saul, sp.nov., holotype SDSNH 67066, SDSNH loc. 4105, Vista area, California, height 14.7 mm, ×2.2. Figure 31. Apertural view. Figure 32. Lateral view (uncoated). Figure 33. Abapertural view (uncoated). Figure 34. Apical view (uncoated).



interspaces than elsewhere. Spiral ribs minutely beaded on ramp and near base of body whorl. Aperture large, quadrate. Outer lip flared, smooth. Outer lip interior with approximately 17 evenly spaced teeth not extending to outer lip periphery, but extending a short distance interiorly; eight medial teeth strongest, others become increasingly weaker posteriorly or anteriorly; teeth tend to align with spiral ribs on exterior of shell. Inner lip with eight teeth; two posteriormost ones strongest (tooth 1 removed from being the posteriormost the strongest), next three teeth slightly weaker, and anteriormost three the weakest. Deck area sharply demarcated from shell; broad and callused, with about 12 very loosely arranged, transverse rows of low ridges or wrinkles (on posterior part of deck and coincident with spiral ribs) and elongate nodes (on anterior part of deck) somewhat coincident with inner lip teeth. Original color pattern with alternating collabral bands of light and dark, with zigzag borders. Growth lines prosocline.

**Dimensions of holotype:** Height 14.7 mm, width 18 mm.

**Holotype:** SDSNH 67066.

**Type locality:** SDSNH 4105, 33°09'45"N, 117°12'37"W, Santiago Formation, near Vista, northern San Diego County, southern California.

**Other material examined:** Two specimens from SDSNH loc. 3522.

**Distribution:** Santiago Formation near Vista, northern San Diego County, southern California (SDSNH locs. 3522 and 4105).

**Geologic age:** Late early to early middle Eocene ("Domengine Stage").

**Discussion:** Three specimens were found. A complete and exceptionally well preserved one (holotype) is from SDSNH loc. 4105, which is from the same general location of UCR loc. 4865 reported by Givens & Kennedy (1976). They reported that the mollusks at UCR loc. 4865 are indicative of the "Domengine Stage" and that they lived in a low-energy, very shallow (0–30 m) brackish-water or marine environment, perhaps a lagoon or estuary.

The other two specimens of the new species are partial specimens from SDSNH loc. 3522. The mollusks at this latter locality are also indicative of the "Domengine Stage" and lived in a brackish-marine lagoon and were transported a short distance seaward and concentrated within a channel complex, along with land-plant remains (Squires, 1992).

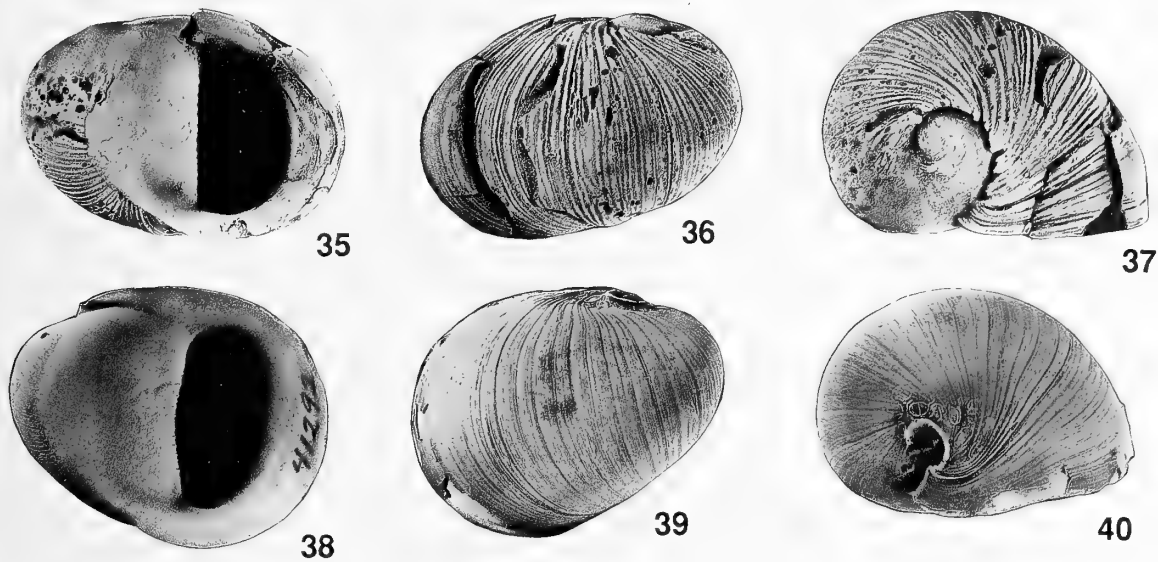
*Theliostyla* probably originated in the Old World Tethyan paleobiotic province and immigrated to the Pacific slope of North America during the late Paleocene. The earliest record of this subgenus in the rock record of the northeastern Pacific is *Nerita* (*Theliostyla*) n. sp.? Woods

& Saul, 1986, of probable late Paleocene age in Baja California. Ascending biostratigraphically, the other known species of *Theliostyla*, besides the new species, from the Pacific slope of North America are the following: "Capay Stage" *Nerita* (*Theliostyla*) *olympia* Squires & Goedert, 1994, from southwestern Washington; "Capay Stage" through "Tejon Stage" *Nerita* (*Theliostyla*) *triangulata* Gabb, 1869 (see previous discussion) from widespread localities; "Tejon Stage" *Nerita* (*Theliostyla*) *crooki* Clark, 1938, from northern California; middle Miocene *Nerita* (*Theliostyla*) sp. from southern California (Susuki, 1978); middle Miocene *Nerita* (*Theliostyla*?) *joaquinensis* Addicott, 1970, from central California; and Pleistocene to Recent *Nerita* (*Theliostyla*) *funiculata* Menke, 1851 [invalid synonym: *Nerita* (*Theliostyla*) *bernhardi* Récluz, 1850] from Pleistocene rocks in Baja California (Durham, 1950) and living in warm waters of Baja California through the Gulf of California and southward to Peru and the Galápagos Islands (Keen, 1971).

*Theliostyla* is normally characterized by granules (pustules) on the deck area. On some species, both fossil and modern, however, there can be considerable variability in the shape of the granules. Specimens of *Nerita* (*T.*) *triangulata* provide an Eocene example. Examination of 43 well preserved late juvenile to adult individuals from SDSNH loc. 4105 revealed a gradation (independent of size) from specimens having only well developed granules on the callus (many specimens) to those having only wrinkles on the callus (few specimens). In some cases, the wrinkles are coincident with spiral ribs, just as on the new species. Specimens of *Nerita* (*T.*) *funiculata* provide a modern example. Examination of about 100 juvenile to early adult individual specimens collected by R. L. Squires from Bahía de Los Angeles in the Gulf of California revealed a gradation (independent of size) from specimens having predominantly granules on the callus (most specimens) to those having only wrinkles on the callus (few specimens). Again, the wrinkles are usually coincident with spiral ribs. Some specimens of *N. (T.) funiculata* even have an almost smooth-deck callus.

The deck area of the new species is known only from the holotype. Although its deck does not have the completely granular ornament that is commonly found in specimens of *Theliostyla*, it could be argued that the new species is within the range of morphology found within the subgenus. However, until specimens, found by future collecting, show the presence of granules on the deck area, it seems prudent to questionably assign the new species to *Theliostyla*.

The new species is very similar to those specimens of *Nerita* (*Theliostyla*) *triangulata* Gabb (1869:170, pl. 28, figs. 52, 52a) that have no carinae on the late part of the body whorl. Squires, (1992:323–329, figs. 1–18) reviewed *N. (T.) triangulata*, a moderately common gastropod in lower and middle Eocene rocks of the Pacific slope of North America. Based primarily on specimens from



Figures 35–40. All specimens coated with ammonium chloride. Figures 35–37. *Neritina (Neritina?) martini* Dickerson, 1915, holotype CAS 291, CAS loc. 193, Vader area, Washington, height 18 mm,  $\times 1.7$ . Figure 35. Apertural view. Figure 36. Abapertural view. Figure 37. Apical view. Figures 38–40. *Neritina (Neritina) pulligera* Linnaeus, 1766, hypotype LACM 152685, Tjilatjap, Java, height 24 mm,  $\times 1.5$ . Figure 38. Apertural view. Figure 39. Abapertural view. Figure 40. Apical view.

SDSNH loc. 3522, which is one of the localities where the new species was found, Squires found that most specimens of *N. (T.) triangulata* have three carinae on the body whorl, some specimens show a gradation from three strong carinae on the early part of the body whorl to faint carinae or no carinae on the late part of the body whorl. The new species differs from *N. (T.) triangulata* by having no carinae whatsoever on the early part of the body whorl. In addition, the new species differs in the following ways: larger and more globose, slightly stronger spiral ribs, much less beaded spiral ribs, and color pattern arranged in collabral bands. Associated with the new species at SDSNH loc. 4105 are abundant and exceptionally well preserved specimens of *N. (T.) triangulata*.

The new species is also very similar to *Nerita (The-liostyla) crooki* Clark (1938:700, pl. 4, figs. 1, 2) from the middle Eocene ("Tejon Stage") Markley Formation, northern California. The new species differs from *N. (T.) crooki* by having mostly unbeaded spiral ribs and by having weaker teeth on the posterior part of the inner lip.

The nomenclature of the formation that contains the type locality of the new species has been in a state of flux in recent years. Givens & Kennedy (1976) referred to the strata as unnamed. Eisenberg & Abbott (1991) assigned the strata to the Delmar Formation, and Squires (1992) followed this assignment. Walsh (1996) assigned the strata to the Santiago Formation, and this usage is followed in this paper.

**Etymology:** The species is named for George L. Ken-

nedy, who informed the authors about the specimens of the new species.

#### Genus *Neritina* Lamarck, 1816

**Type species:** *Nerita pulligera* Linnaeus, 1766 (ICZN opin. 119, 1931); Recent, southwest Pacific.

#### Subgenus *Neritina sensu stricto*

**Discussion:** *Neritina sensu stricto* is low spired and has a smooth or finely dentate inner lip. It has an outer lip that overrides the body whorl and forms a projecting point in the spire area. It also has a very slightly sinuous inner lip (Keen & Cox, 1960) (Figures 37–39). The holotype of *Neritina martini* Dickerson, 1915, discussed below, does not have this projecting point, and its absence is most likely because of poor preservation of this very delicate feature or because of abrasion. The holotype of *N. martini* also has a straight inner lip, and this difference, along with a lack of information about the projecting point, makes the subgeneric assignment of *N. martini* tentative.

#### *Neritina (Neritina?) martini* Dickerson, 1915

(Figures 35–37)

*Neritina martini* Dickerson, 1915:59, pl. 5, figs. 8a,b; Weaver, 1943:296, pl. 63, fig. 10.

**Holotype:** CAS 291.

**Type locality:** CAS loc. 183, Cowlitz Formation, Lewis County, southwestern Washington.

**Other material examined:** None.

**Distribution:** Cowlitz Formation, Lewis County, southwestern Washington (CAS loc. 183).

**Geologic age:** Late middle Eocene ("Tejon Stage").

**Discussion:** This species is known only from the holotype, which is an adult specimen (height 18 mm, diameter 21 mm) that is well preserved, except for the apical area. Dickerson's (1915:pl. 5, fig. 8a) illustration of the apertural view of the holotype of *N. (N.) martini* shows the inner lip, whereas Weaver's (1943:pl. 63, fig. 10) illustration of the same view of this specimen shows the aperture plugged with modeling clay.

*Neritina* is a littoral zone or fresh-to brackish-water gastropod (Fürsich & Kauffman, 1984). Its presence in the Cowlitz Formation is extremely rare, but is compatible with the deltaic setting of the formation. Contemporaneous environments on this delta included brackish-water areas in mudflats and marshes, as well as a freshwater lake within the marshes, all in close proximity to near-shore-marine habitats (Nesbitt, 1995).

Cossmann (1925) reported the geologic range of *Neritina* (*Neritina*) as Middle Jurassic to Recent, whereas Keen & Cox (1960) reported it as Recent only. The latter workers, however, did report the geologic range of *Neritina sensu lato* as Eocene to Recent. Inconsistencies such as these are a reflection of the poor state of knowledge of this group of neritids, which is represented by a paucity of well preserved specimens. Without adequate information about the inner lip and deck area, workers have been understandably uncertain about the identification of the specimens.

Although early workers reported several species of *Neritina* and *Neritina?* from Jurassic and Cretaceous rocks of the western interior of the United States (see Boyle, 1893, for a summation), nearly all of these species subsequently have been re-evaluated and assigned to other genera (e.g., Yen, 1946, 1951; Sohl, 1965; Fürsich & Kauffman, 1984). At least two species have been retained in *Neritina*; namely, *Neritina insolita* Stephenson (1952: 146, pl. 54, figs. 6–8) from the Upper Cretaceous (Cenomanian Stage) Woodbine Formation of Texas and *Neritina* sp. (Dockery, 1993) from Upper Cretaceous (Maastrichtian Stage) strata in Mississippi.

The species of *Neritina* from Paleocene and Eocene rocks of Paris Basin, France have also been reassigned to other genera (Le Renard & Pacaud, 1995:90). Furthermore, it seems to us that the lower Eocene *Neritina unidentata* Aldrich (1911:13, pl. 5, figs. 7, 8), which is the only reported species of *Neritina* from the Paleogene of the Gulf coast of the United States, should be placed in genus *Neritoplica* Oppenheim, 1892, based on the overall

shape of the shell and the presence of a single projecting tooth on the inner lip.

An exhaustive study of all fossil occurrences of *Neritina* is beyond the scope of this present investigation, but our rudimentary review of the literature indicates that *Neritina sensu lato* is a rare taxon whose earliest known record is probably the early Late Cretaceous (Cenomanian).

In addition to *Neritina* (*N.?*) *martini*, the only fossil record of *Neritina* on the Pacific slope of North America includes *Neritina* (*Dostia*) *cuneata* (Gabb, 1864) from Upper Cretaceous (Campanian Stage) strata of northern California and *N. (D.)* aff. *N. (D.) cuneata* (Gabb) of Woods & Saul, 1986, which is a very similar, if not conspecific form, from the Upper Cretaceous (Maastrichtian Stage) Tierra Loma Sandstone Member of the Moreno Formation of north-central California (Woods & Saul, 1986). *Dostia* Gray, 1842, is patelliform with seven to nine ridgelike teeth and is morphologically quite distinct from *Neritina sensu stricto*.

The only modern record of *Neritina* on the Pacific slope of North America is *Neritina* (*Clypeolum*) *latissima* Broderip, 1833, known from Acapulco, Mexico to Ecuador (Keen, 1971). *Clypeolum* Récluz, 1842, has a large flaring aperture and is morphologically quite distinct from *Neritina sensu stricto*.

*Neritina* (*N.?*) *martini* is most like *Neritina* (*Neritina*) *pulligera*, a modern species and the type species of *Neritina* (*Neritina*). Illustrations of this type species are provided in Figures 38–40. *Neritina* (*N.?*) *martini* differs from *N. (N.) pulligera* by having a more elliptical shape, straight inner lip, and more incised growth lines. As mentioned earlier, whether or not *Neritina* (*N.?*) *martini* has an outer lip that overrides the body whorl as a projecting point in the spire area cannot be determined.

One other North American fossil species of *Neritina* has been compared to *Neritina* (*N.?*) *pulligera*. Stephenson (1952) reported *Neritina insolita* Stephenson from the Woodbine Formation (Cenomanian) of Texas to be very similar to *Neritina pulligera*. *Neritina* (*N.?*) *martini* and *N. insolita* are also similar and both have a straight inner lip, but the former differs from *N. insolita* by having denticles on the inner lip and having no spiral ribs on the shell.

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- LACMIP 9936 [= LACMIP 28787]. Fossiliferous brown sandstone about 4.5 km (2.8 mi.) S of U.S. Highway 26, along west side of Bridge Creek, 610 m (2000 ft.) N and 805 (2640 ft.) E of SW corner of section 25, T. 13 S, R. 27 E, 44°24'34"N, 119°25'10"W, U.S. Geological Survey, 7.5-minute, Aldrich Mtn. North Quadrangle, 1972 (photorevised 1983), Grant County, east-central Oregon. Unnamed strata. Age: Earliest Late Cretaceous (Cenomanian Stage). Collectors: W. P. Popenoe and J. Alderson, June 12, 1975.
- LACMIP 10508. At approximately 404 m (1325 ft.) elevation, just below a coralline-algal interval in limey, muddy siltstone and west of small fault, in roadcut on north side of dirt road, on north slope of Trailer Canyon near top of ridge between Quarry and Trailer canyons, at approximately 50 m east of steel gate at boundary of Topanga State Park, and 4435 m (14,547 ft.) S and 5334 (17,496 ft.) W of NE corner of U.S. Geological Survey, 7.5-minute, Topanga Quadrangle, 1952 (photorevised 1981), east-central Santa Monica Mountains, Los Angeles County, southern California. Upper part of Santa Susana Formation. Age: Late Paleocene ("Martinez Stage"). Collectors: R. L. Squires and students, 1997.
- LACMIP 10676 [= CIT 1559]. Conglomeratic and fossiliferous outcropping in bed and banks of Los Banos Creek, 823 m (2700 ft.) N and 305 m (1000 ft.) W of SE corner of section 12, T. 11 S, R. 9 E, 36°59'28"N, 120°55'50"W, U.S. Geological Survey, 7.5-minute, Ortigalita Peak NW Quadrangle, 1969 (photorevised 1984), Merced County, north-central California. Moreno Formation, "Quinto Shale" member. Age: Late Cretaceous (Maastrichtian Stage). Collectors: B. C. Adams, R. W. Burger & L. Simon, circa 1942. [Locality is now at damsite of the Los Banos Reservoir.]
- LACMIP 10685 [= CIT 1573]. 975 m (3200 ft.) N and 549 m (1800 ft.) W of SE corner of section 12, T. 11 S, R. 9 E, 36°59'03"N, 120°55'58"W, U.S. Geological Survey, 7.5-minute, Ortigalita Peak NW Quadrangle, 1969 (photorevised 1984), Merced County, north-central California. Moreno Formation, "Quinto Shale" member. Age: Late Cretaceous (Maastrichtian Stage). Collectors: B. C. Adams & W. P. Popenoe, 1942. [Locality is now along the eastern side of the Los Banos Reservoir.]
- LACMIP 22536. See LACMIP 6298.

## APPENDIX

## LOCALITIES CITED

- CAS 183. See LACMIP loc. 6298.
- LACMIP 6297. In west bank of "Big Bend" of Cowlitz River, 590 m (1935 ft.) N and 375 m (1230 ft.) W of SE corner of section 28, T. 11 N, R. 2 W, U.S. Geological Survey, 15-minute, Castle Rock Quadrangle, 1953, about 2 km (1.2 mi.) E of Vader, Lewis County, southwestern Washington. Cowlitz Formation. Age: Late middle Eocene ("Tejon Stage"). Collectors: J. L. Goedert, 1982; R. L. Squires, July 13, 1988.
- LACMIP 6298 [= CAS 183, LACMIP 22536 (= UCLA 2536), UWBM 232, & UWBM 329]. Up-river from LACMIP loc. 6297, in west bank of "Big Bend" of Cowlitz River, 725 m (2378 ft.) N and 285 m (935 ft.) W of SE corner of section 28, T. 11 N, R. 2 W, U.S. Geological Survey, 15-minute, Castle Rock Quadrangle, 1953, about 2 km (1.2 mi.) E of Vader, Lewis County, southwestern Washington. Cowlitz Formation. Age: Late middle Eocene ("Tejon Stage"). Collectors: Numerous workers over the last 80 years or so.
- LACMIP 7047. A thin but richly fossiliferous layer of



- LACMIP 23348 [= UCLA 3348]. At elevation of 152 m (500 ft.), in light gray, fine-grained calcareous cemented arkosic sandstone, 30 to 60 cm thick, rich in molluscan fossils, in a small tributary (upper part of Well Canyon), north of Cañada Posa, 4325 m (14,190 ft.) N and 1737 m (5700 ft.) W of SE corner of U.S. Geological Survey, 7.5-minute, Santa Cruz Island A Quadrangle, 1943, southwestern Santa Cruz Island, Channel Islands, Santa Barbara County, southern California. Pozo Formation. Age: Late Paleocene ("Martinez Stage"). Collector: T. Rothwell, February 4, 1955.
- LACMIP 26720 [= UCLA 6720]. Hill with firebreak, 213 m (700 ft.) E of where 358 m (1175 ft.) contour line crosses Pulga Canyon, just below massive algal limestone beds, U.S. Geological Survey, 7.5-minute, Topanga Quadrangle, 1952 (photorevised 1981), Palisades Highlands, Santa Monica Mountains, Los Angeles County, southern California. Upper part of Santa Susana Formation. Age: Late Paleocene ("Martinez Stage"). Collector: J. Alderson, October, 1980.
- LACMIP 27083 [= UCLA 7083]. On a NW-facing hill-slope 7.1 km (4.4 mi.) ESE of Punta Rosarito and 0.9 km (0.6 mi.) E of Bahía Sebastian Vizcaino, southwestern Baja California Norte Mexico. Sepultura Formation. Age: Probably late Paleocene ("Martinez Stage"). Collector: A. J. C. Woods, circa 1975.
- SDSNH 3522. At elevation of 174 m (570 ft.), from a temporary excavation of approximately 7.5 m (24.6 ft.) of interbedded sandstone and muddy siltstone within a channel complex at the Laurels housing project, 372 m (1220 ft.) N and 665 m (2180 ft.) E of the SW corner of section 17, T. 12 S, R. 3 W, U.S. Geological Survey, 7.5-minute, San Marcos Quadrangle, 1968 (photorevised 1983), Laurels housing development project, west of the city of San Marcos, northern San Diego County, southern California. Santiago Formation. Age: Middle Eocene. Collector: D. J. McGuire, November 30, 1989.
- SDSNH 4105. From excavation on south side of California State Highway 78, in upper part of a medium dark gray, silty, fine-grained sandstone about 2.1 m (7 ft.) thick, about 3 km (1.86 mi.) SE of Sycamore Avenue, city of Vista, U.S. Geological Survey Quadrangle, 7.5-minute, San Marcos Quadrangle, 1968 (photorevised 1983), northern San Diego County, southern California. Santiago Formation. Age: Middle Eocene. Collectors: B. O. Riney and S. L. Walsh, August 12, 1996. [Excavation has been covered by backfilling of a new concrete retaining wall along south side of freeway.]
- UCR 4865. In roadcut on south side of California State Highway 78, 5.2 km (3.2 mi.) SE of city of Vista and 4.8 km NW of city of San Marcos, U.S. Geological Survey Quadrangle, 7.5-minute, San Marcos Quadrangle, 1968 (photorevised 1983), northern San Diego County, southern California. Santiago Formation. Age: Middle Eocene. Collector: C. R. Givens.
- UWBM 232. See LACMIP loc. 6298.
- UWBM 329. See LACMIP loc. 6298.



## Review of the Genus *Actinocyclus* Ehrenberg, 1831 (Opisthobranchia: Doridoidea)

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**Abstract.** The genus *Actinocyclus* comprises two species, *Actinocyclus verrucosus* Ehrenberg, 1831, which is widespread through the tropical Indo-Pacific, from the Red Sea to Australia and Hawaii, and *Actinocyclus papillatus* (Bergh, 1878) known from East Africa, Papua New Guinea, the Philippines, and Japan. These two species are distinguishable by the external morphology and the arrangement and shape of the reproductive organs. There are no major radular differences. The genus name *Spaerodoris* is a junior synonym of *Actinocyclus*. Other species names described within these two genera are either junior synonyms of *A. verrucosus* (*Spaerodoris punctata* Bergh, 1878, *Spaerodoris laevis* Bergh, 1890, *Spaerodoris japonica* Eliot, 1913), synonyms of *A. papillatus* (*Spaerodoris laevis* var. *variegata* Eliot, 1904), or belong to different genera (*Actinocyclus fragilis* Ehrenberg, 1831, *A. velutinus* Ehrenberg, 1831). *Aldisa nhatrangensis* Risbec, 1956, is also a junior synonym of *Actinocyclus verrucosus*.

### INTRODUCTION

Gosliner & Johnson (1994) studied the phylogenetic relationships of the genus *Hallaxa* Eliot, 1909, and hypothesized that *Actinocyclus* Ehrenberg, 1831, was its sister taxon. According to these authors, *Actinocyclus* and *Hallaxa* are the only two members of the family Actinocyclusidae, which is the sister taxon to the Chromodorididae. At that point, the anatomy of *Actinocyclus* was known only from drawings of the reproductive system of *A. japonicus* by Kay & Young (1969) and Gosliner & Johnson (1994), and drawings of several radular teeth by Kay & Young (1969).

Up to now, several names have been proposed for species of this genus, but no one knows for sure how many valid species it comprises. Most authors appear to agree that *A. japonicus* is the valid name for a widespread Indo-Pacific species (Kay & Young, 1969; Bertsch & Johnson, 1981; Willan & Coleman, 1984; Wells & Bryce, 1993; Gosliner & Johnson, 1994), but its relationships with the type species, *A. verrucosus* Ehrenberg, 1831, from the Red Sea, are unknown.

The position of the Actinocyclusidae at the base of the Cryptobranchia makes this group essential for further understanding of the phylogenetic relationships within this diverse clade of dorids. Therefore, a more detailed knowledge of the anatomy of this taxon is critical for future research on the phylogeny of the Cryptobranchia.

In this paper I attempt a comprehensive anatomical study of the genus *Actinocyclus*, including features that might be important for future phylogenetic research, such as the central nervous system, the digestive system, and the reproductive system. In addition, a systematic review

of all species described is carried out in light of examination of specimens collected from several Indo-Pacific localities, trying to cover the entire geographic range of *Actinocyclus*. I also attempted to locate in several natural history museums the type material and other historically important specimens of all nominal species involved.

The material examined is deposited at the Department of Invertebrate Zoology and Geology of the California Academy of Sciences, San Francisco (CASIZ), the Museum für Naturkunde der Humboldt-Universität zu Berlin (MHUB), the Zoologisk Museum, Københavns Universitet, Copenhagen (ZMUC), and the Muséum National d'Histoire Naturelle, Paris (MNHN).

### SYSTEMATIC DESCRIPTIONS

#### Genus *Actinocyclus* Ehrenberg, 1831

*Actinocyclus* Ehrenberg, 1831: [28]. Type species: *Actinocyclus verrucosus* Ehrenberg, 1831, by subsequent designation of J. E. Gray (1847).

*Spaerodoris* Bergh, 1877:66. Type species: *Actinocyclus verrucosus* Ehrenberg, 1831, by monotypy.

**Remarks:** Ehrenberg (1831) introduced the new genus *Actinocyclus* based on two briefly described new species: *Actinocyclus verrucosus* and *A. velutinus*. A third species, *Actinocyclus fragilis*, was included with a question mark. Subsequently, J. E. Gray (1847) selected *Actinocyclus verrucosus* as the type species of *Actinocyclus*.

Bergh (1877) introduced the new genus *Spaerodoris* based on *Actinocyclus verrucosus* Ehrenberg, 1831, including two new, not yet described species from the Philippines, and with a question mark "*Doris incii* (Alder)" [*Doris incii* J. E. Gray in M. E. Gray, 1850 is probably

a senior synonym of *Halgerda willeyi* Eliot, 1903 (S. Fahay, personal communication)]. Bergh (1877: 66–67) commented that *Actinocyclus* and *Sphaerodoris* are probably synonyms, but anatomical studies on *Actinocyclus* would be necessary to confirm this point. Since *Actinocyclus* was not anatomically studied, Bergh regarded this name as a synonym of *Sphaerodoris*. These sorts of decisions, which clearly violated the law of priority, were often taken by Bergh to suppress poorly described taxa. In any case, *Actinocyclus verrucosus* is the only valid nominal species originally and without doubt included in the genus *Sphaerodoris* Bergh, 1877, and therefore it is the type species by monotypy. Thus, *Sphaerodoris* is a junior objective synonym of *Actinocyclus*.

Eliot (1913) re-examined the holotype of *A. verrucosus* and confirmed that it belonged to the same genus as Bergh's species of *Sphaerodoris*. He also considered that *Actinocyclus* should be suppressed because of several contradictions between the original description and the actual specimen. On the contrary, O'Donoghue (1929) recognized that *Actinocyclus* is a valid genus and thus a senior synonym of *Sphaerodoris*. At the same time, he designated *Sphaerodoris punctata* as the type species of *Sphaerodoris*, but since this species was not included in the original description, such a designation is not valid. Since O'Donoghue's paper, most authors have accepted the name *Actinocyclus* as valid, and it is currently in common usage (Kay & Young, 1969; Bertsch & Johnson, 1981; Willan & Coleman, 1984; Wells & Bryce, 1993; Gosliner & Johnson, 1994).

Gosliner & Johnson (1994) reviewed the differences between *Hallaxa* and *Actinocyclus*. In light of phylogenetic analysis they concluded that these two genera are clearly distinguishable by the presence of two apomorphic features in *Actinocyclus*: "an elaboration of the anterior border of the foot and the presence of a secondary ampulla next to the hermaphroditic duct," and one apomorphic feature in *Hallaxa*: "presence of an elongate prostatic portion of the vas deferens." The present study confirms the presence of the elaboration of the anterior border of the foot and an unusual ampulla in *Actinocyclus*. In most groups of dorid nudibranchs the ampulla appears to be a dilatation of the hermaphroditic duct, whereas in species of *Actinocyclus* it is a lateral expansion. Even though the ampulla of *Actinocyclus* is arranged differently from that of other dorids, it seems to be a homologous structure and the term "secondary ampulla" appears not to be appropriate.

*Actinocyclus verrucosus* Ehrenberg, 1831

(Figures 1A,B, 2–4)

*Actinocyclus verrucosus* Ehrenberg, 1831:[28–29].

*Sphaerodoris punctata* Bergh, 1877:66 (*nomen nudum*).  
Bergh, 1878:587–590, pl. 65, figs 1–5.

*Sphaerodoris laevis* Bergh, 1890:925–928, pl. 88, figs 3–12.

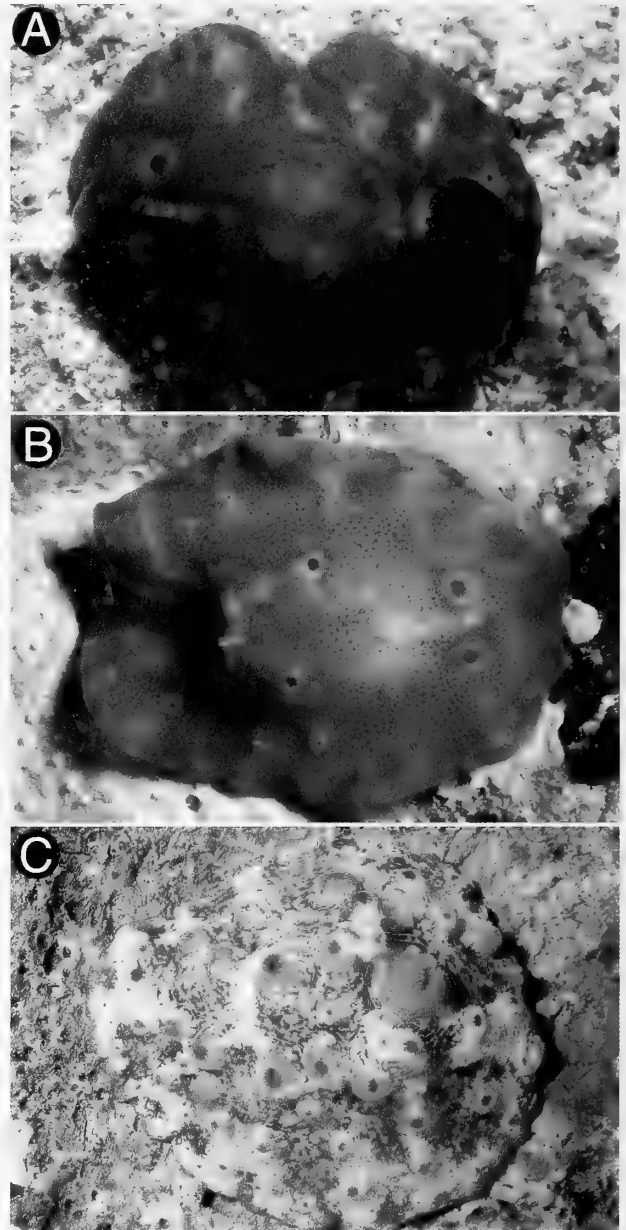


Figure 1. Living animals. A. Specimen of *Actinocyclus verrucosus* from Madagascar (CASIZ 073553), photo by T. M. Gosliner. B. Specimen of *A. verrucosus* from the Philippines (CASIZ 083843), photo by T. M. Gosliner. C. Specimen of *Actinocyclus papillatus* from Papua New Guinea (CASIZ 068651), photo by T. M. Gosliner.

*Sphaerodoris japonica* Eliot, 1913:23–26.

*Aldisa nhatrangensis* Risbec, 1956:14–15, pl. 20, fig. 109,  
pl. 22, upper right figure.

**Type material:** *Actinocyclus verrucosus* Ehrenberg, Holotype (by monotypy): "Massaua" = Mits'iwa Island, Ethiopia, dissected (MHUB 594).

*Sphaerodoris punctata* Bergh. Holotype (by monotypy).

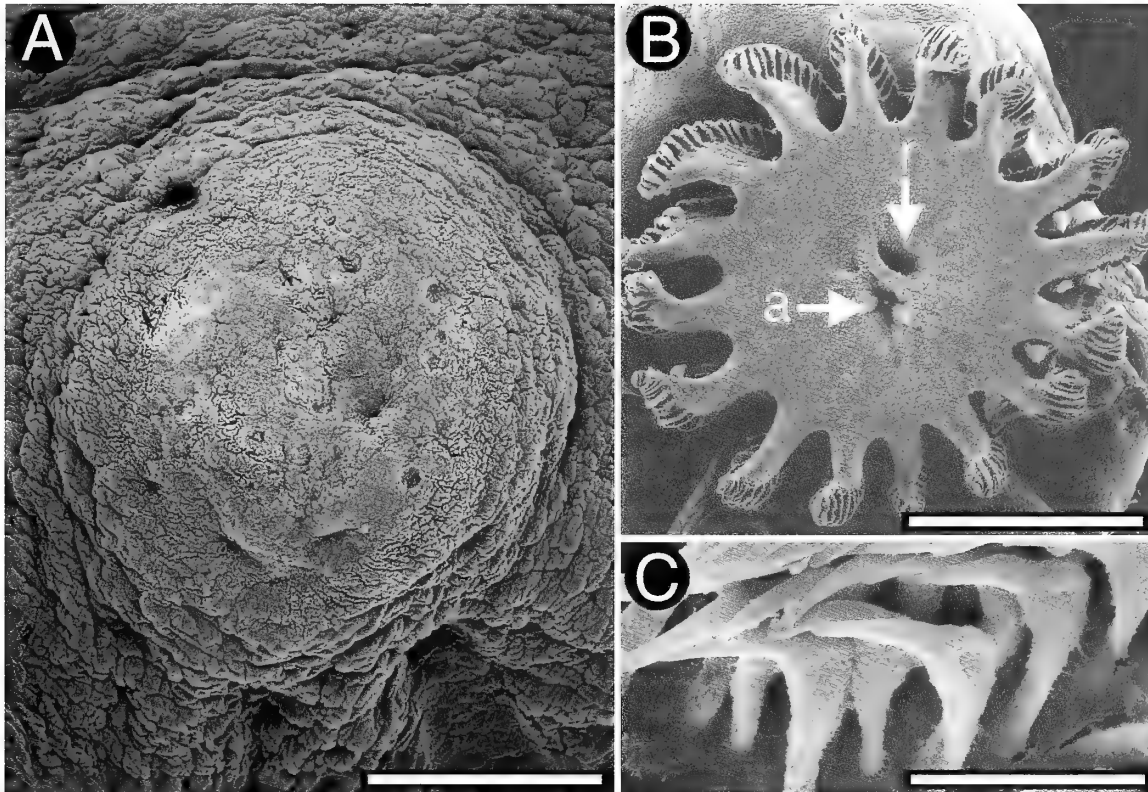


Figure 2. *Actinocyclus verrucosus*, scanning electron micrographs. A. Dorsal tubercle (CASIZ 099250), scale bar = 600 µm. B. Gill (CASIZ 086635), scale bar = 1.5 mm. C. Jaw elements (CASIZ 073553), scale bar = 15 µm. Abbreviations: a, anus opening; r, renal opening.

py): “Lapinig” probably Lapinin Island, Northwest of Bohol Island, Philippines, dissected (ZMUC GAS-2118).

*Aldisa nhatrangensis* Risbec. Holotype (by original designation): Hon Lon, Nha Trang, Vietnam, dissected (MNHN).

The type material of *Spaherodoris laevis* Bergh, and *Spaherodoris japonica* Eliot, is probably lost. No specimens are deposited at the Zoologisk Museum, Københavns Universitet, Copenhagen (K. Jensen, personal communication) or The Natural History Museum, London (A. Campbell, personal communication).

**Additional material:** South of Soanambo Hotel, Île Saint Marie, Madagascar, 6 April 1990, 1 specimen 18 mm preserved length, collected by T. M. Gosliner (CASIZ 073553). Devil’s Point, southwest side of Maricaban Island, Batangas, Luzon, Philippines, 19 February 1992, 1 specimen 21 mm preserved length, collected by T. M. Gosliner (CASIZ 083843); 19 February 1992, 1 specimen 39 mm preserved length, collected by T. M. Gosliner (CASIZ 083793); 17 March 1994, 1 specimen 20 mm preserved length, 24 m depth, collected by T. M. Gosliner (CASIZ 096290); 15 April 1996, 2 specimens 21 and 23 mm preserved length, 20 m depth, collected by T. M. Gosliner (CASIZ 106456). Okinawa, Ryukyu Islands, Ja-

pan, 2 May 1992, 1 specimen 23 mm long, dissected, collected by R. Bolland (CASIZ 086635). Tengan Pier, 14 km West of Ikei-shima, Okinawa, Ryukyu Islands, Japan, 9 April 1994, 1 specimen 35 mm preserved length, 12 m depth, dissected, collected by R. Bolland (CASIZ 099250), 18 October 1994, 1 specimen 33 mm preserved length, 12 m depth, collected by R. Bolland (CASIZ 104697). O Ennubuj, Kwajalein Atoll, Marshall Islands, 6 December 1992, 1 specimen 24 mm preserved length, 6 m depth, collected by S. Johnson (CASIZ 116662). Makua, Oahu Island, Hawaii, 17 April 1985, 1 specimen 12 mm preserved length, 5 m depth, collected by S. Johnson (CASIZ 116894).

**Geographic range:** This species is known from the Red Sea (Ehrenberg, 1831), East Africa (Eliot, 1904), Vietnam (Risbec, 1956), Japan (Eliot, 1913), the Philippines (Bergh, 1878), Indonesia (Bergh, 1890), Malaysia (Eliot, 1904), Western Australia (Wells & Bryce, 1993), Queensland (Willan & Coleman, 1984), and Hawaii (Kay & Young, 1969). The present paper reports the first records from Madagascar and the Marshall Islands.

**External morphology:** The body is elevated, short, oval, almost as long as wide (Figures 1A, B). The dorsum is

covered with several simple conical tubercles scattered irregularly. Some of them are much larger and have a depression on the apex (Figure 2A). The central part of the body is clearly elevated over the mantle margin, which is relatively narrow. The perfoliate rhinophores are composed of 20 lamellae in a 33 mm-long specimen (CASIZ 104697). There are 16 unipinnate branchial leaves in the 33 mm-long specimen (Figure 2B). In the living animal they are pointing inward, with the apices very close to each other.

The background color varies from cream brownish to dark brown or gray (Figure 1B). Some specimens are almost black (Figure 1A). There are paler areas, generally white or yellowish, irregularly distributed on the dorsum. The entire dorsum is covered with small, evenly distributed black spots. The depressions on the tips of the larger tubercles are dark brown or black. The gill is dark gray or black, with numerous small white spots, more densely distributed near the base. The rhinophores are the same color as the body.

The anterior border of the foot is not grooved or notched, with anteriorly directed foot margins partially surrounding the mouth area (Figure 3F). There are no oral tentacles.

**Anatomy:** The posterior end of the glandular portion of the oral tube has two strong retractor muscles (Figure 3D) which attach to the body wall. The oval, muscular buccal bulb has four additional muscles attached. Two long salivary glands connect with the buccal bulb at the sides of the esophageal junction. The buccal bulb is as long as the glandular portion of the oral tube. The jaws are composed of numerous undivided rodlets (Figure 2C). The radular formula is  $65 \times 15.0.15$  in a 35 mm-preserved-length specimen (CASIZ 099250) and  $70 \times 21.0.21$  in a 18 mm-preserved-length specimen (CASIZ 073553). Rachidian teeth are absent. The innermost lateral teeth are broad and thick (Figures 4A, B). They have a large rounded cusp and bear six to seven denticles along their inner edge. The mid-lateral teeth are narrow basally and elongated (Figure 4C). The outermost teeth are shorter than the mid-laterals (Figure 4D). The outer laterals bear 13–17 denticles along their inner margin. The esophagus is short and convoluted and connects with the digestive gland.

The narrow hermaphroditic duct expands into the elongate and convoluted ampulla, which inserts distally at the junction of the oviduct and the prostate (Figures 3B, C). The oviduct enters the female gland in the center of the mass. The prostate is rounded, as long as wide, and granular. It connects with a long duct that narrows and expands again into the short ejaculatory portion of the deferent duct. The muscular ejaculatory portion opens into a common atrium with the vagina. The penis is unarmed. The vagina is very long and undulate. Near its proximal end it joins the pyriform seminal receptacle, the uterine duct, and the oval bursa copulatrix. Both the bursa co-

pulatrix and seminal receptacle are stalked. The bursa copulatrix is about twice as large as the seminal receptacle (Figure 3C).

In the central nervous system (Figure 3E) the cerebral and pleural ganglia are partially fused and distinct from the pedal ganglia. There is a separate abdominal ganglion on the right side of the visceral loop. Paired gastroesophageal, rhinophoral, and optical ganglia are also present. The pedal ganglia are clearly separated. The pedal and parapedal commissures are fused together.

The circulatory system (Figure 3A) consists of a large heart and a single blood gland situated over the central nervous system.

**Remarks:** The original description of *Actinocyclus verrucosus* is a brief Latin text with no illustrations or anatomical information (Ehrenberg, 1831). The re-examination of the holotype of this species, collected Mits'iwa Island, Ethiopia, confirmed that its identity agrees with the usage of the name *Actinocyclus*. The specimen was dissected by Eliot during the preparation of his 1913 paper, the radula is missing, and the reproductive system is damaged. Yet, the external morphology is identical to that of our specimens.

Bergh (1877) introduced for the first time the name *Spaherodoris punctata*, but without description, and therefore it is a *nomen nudum*. Later, Bergh (1878) described this species based on preserved specimens from the Philippines. It is not possible to confirm their identity based on the original description of the external morphology; however, re-examination of the type material confirms that it is conspecific with *Actinocyclus verrucosus*. The 38 mm-long holotype of this species has the dorsum covered with a few conical tubercles and 21 unipinnate branchial leaves.

*Spaherodoris laevis* was described by Bergh (1890) on the basis of a single specimen collected from Ambon, Indonesia. The preserved animal was described as being uniformly brown or olive gray with some yellowish areas on the dorsum. As described, the radula and reproductive system are identical to those of *Actinocyclus verrucosus*. Unfortunately, the holotype of this species is untraceable, and this synonymy is based on review of the original description.

Eliot (1913) described the new species *Spaherodoris japonica*, from Japan, which, in his opinion, might be conspecific with *A. verrucosus*. According to Eliot (1913), the main difference between *S. japonica* and other species of the genus is that both rhinophores open in the same cavity. However, Eliot recognized that this could be a teratology. He did not find other differences between both species that could justify the separation of *A. japonicus*.

Kay & Young (1969) redescribed *A. japonicus* from Hawaii, and regarded *Aldisa nhatrangensis* Risbec, 1956,

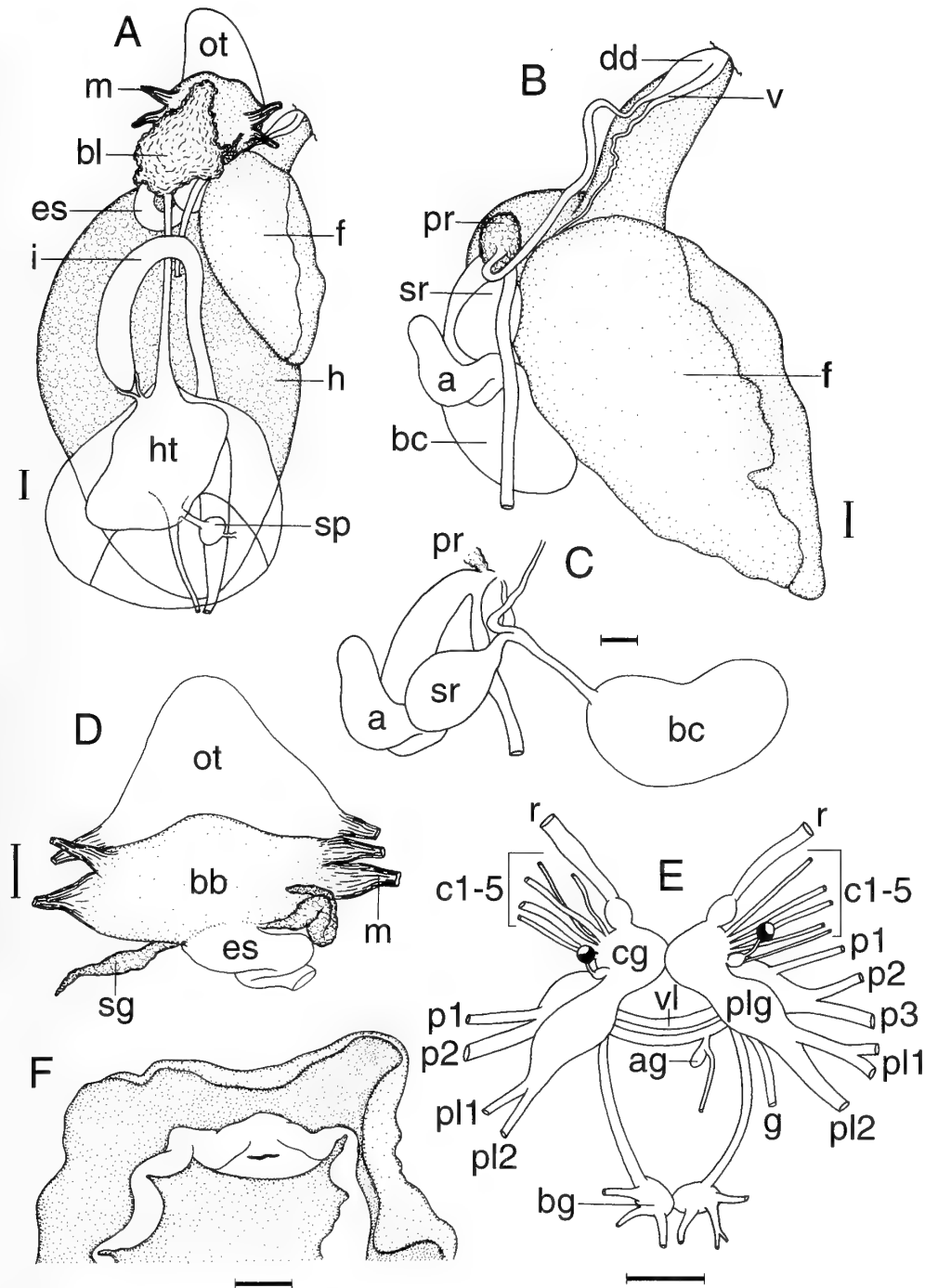


Figure 3. *Actinocyclus verrucosus*, anatomy of a specimen from Japan (CASIZ 099250). A. General view of the anatomy, scale bar = 1 mm. B. Reproductive system, scale bar = 1 mm. C. Detail of several reproductive organs, scale bar = 1 mm. D. Dorsal view of the buccal bulb, scale bar = 1 mm. E. Central nervous system, scale bar = 0.5 mm. F. Ventral view of the mouth area, scale bar = 1 mm. Abbreviations: a, ampulla; ag, abdominal ganglion; bl, blood gland; bc, bursa copulatrix; bg, buccal ganglion; c, cerebral nerves; cg, cerebral ganglion; dd, deferent duct; es, esophagus; f, female glands; g, genital nerve; h, digestive gland; ht, heart; i, intestine; m, retractor muscles; ot, oral tube; p, pedal nerves; pl, pleural nerves; plg, pleural ganglion; pr, prostate; sg, salivary gland; sp, syrinx; sr, seminal receptacle; v, vagina; vl, visceral loop.



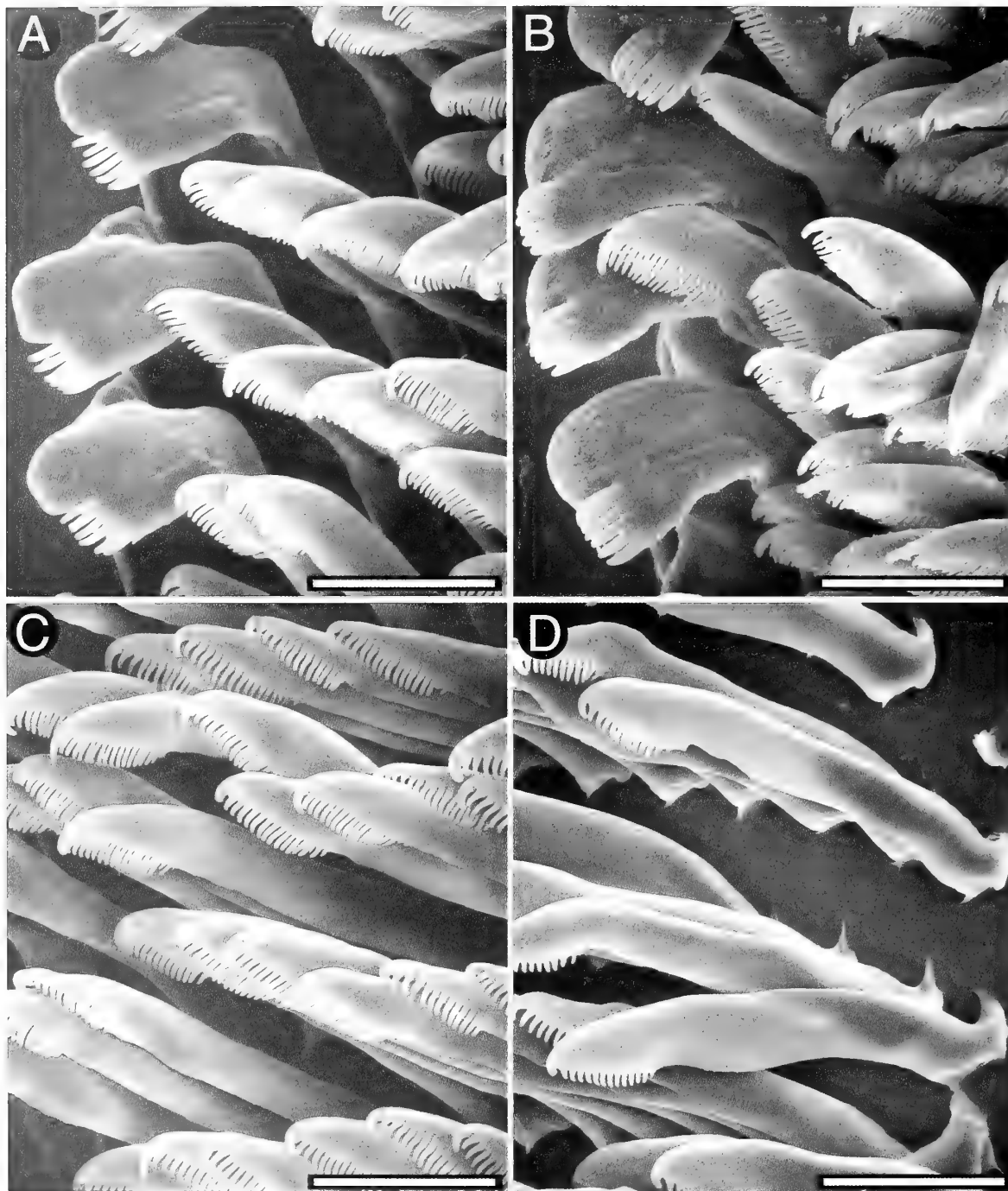


Figure 4. *Actinocyclus verrucosus*, scanning electron micrographs of the radula. A. Innermost lateral teeth of a specimen from Japan (CASIZ 099250), scale bar = 30 µm. B. Innermost lateral teeth of a specimen from Madagascar (CASIZ 073553), scale bar = 25 µm. C. Mid-lateral teeth of a specimen from Japan (CASIZ 099250), scale bar = 30 µm. D. Outermost lateral teeth of a specimen from Japan (CASIZ 099250), scale bar = 30 µm.

as a synonym. The radula and reproductive system are identical to those of our specimens.

After the examination of the type material of several species and additional specimens from Japan, the Philippines, Hawaii, and Madagascar, it is clear that *Actinocyclus verrucosus* is a widespread species in the tropical Indo-Pacific that exhibits wide variation in color, but also a great consistency in reproductive and radular features among different specimens.

There are two other species that were assigned to the genus *Actinocyclus* by Ehrenberg (1831), *Actinocyclus fragilis* Ehrenberg, 1831, and *A. velutinus* Ehrenberg, 1831. Both Bergh (1877) and O'Donoghue (1929) regarded them as unrecognizable. A re-examination of the original description of these taxa reveals that they do not belong to the genus *Actinocyclus* in the sense of its present usage. The dorsum of *A. velutinus* is covered with "very densely arranged minute hairs" (probably caryophyllidia), and *A. fragilis* is a large, yellowish brown species with densely arranged marginal dark spots and numerous dorsal tubercles.

### *Actinocyclus papillatus* (Bergh, 1878)

(Figures 1C, 5,6)

*Sphaerodoris papillata* Bergh, 1877:66 (*nomen nudum*).  
Bergh, 1878:590–592, pl. 66, figs 6,7.

*Sphaerodoris laevis* var. *variegata* Eliot, 1904:403–404.

**Type material:** *Sphaerodoris papillata* Bergh. Holotype (by monotypy): Ubay, Northwest of Bohol Island, Philippines, dissected (ZMUC GAS-2119). The holotype of *Sphaerodoris laevis* var. *variegata* is probably lost. It could not be located in the collections of The Natural History Museum, London (A. Campbell, personal communication).

**Additional material:** Barracuda Point, east side of Pig Island, near Madang, Papua New Guinea, 11 August 1989, 30 m depth, 1 specimen 35 mm preserved length, collected by M. Ghiselin (CASIZ 068651).

**Geographic range:** So far this species is known only from the east coast of Africa (Eliot, 1904), Japan (Hori & Fukuda, 1996), the Philippines (Bergh, 1878), and Papua New Guinea (this paper).

**External morphology:** The body is elevated, short and oval (Figure 1C). The dorsum is covered with numerous simple conical to rounded tubercles scattered irregularly. The tubercles situated on the center of the dorsum and near the gill opening are larger than the others. The central part of the body is clearly elevated over the mantle margin, which is relatively narrow. The perfoliate rhinophores are composed of 22 lamellae. There are 14 unipinnate branchial leaves in the 35 mm-preserved-length specimen. In the living animal they are pointing inward, with the apices very close to each other.

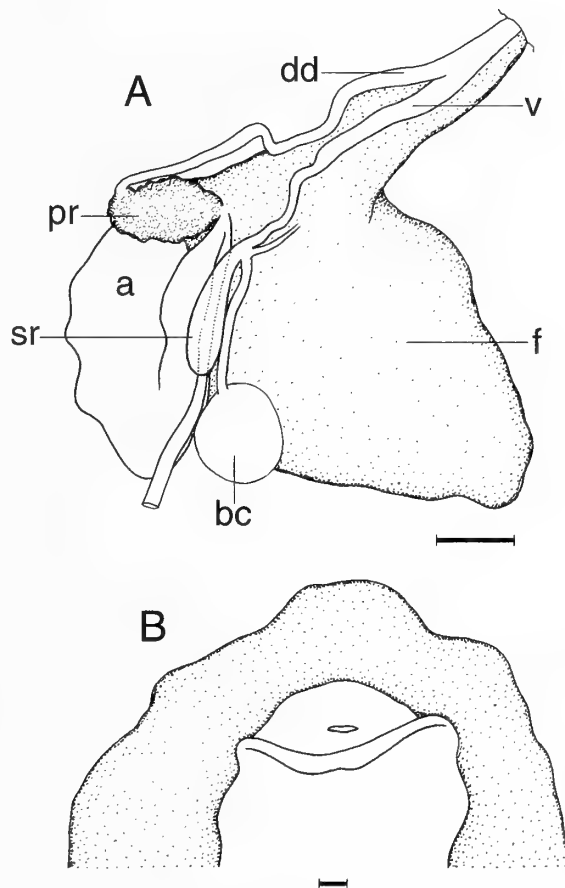


Figure 5. *Actinocyclus papillatus* (CASIZ 068651). A. Reproductive system, scale bar = 1 mm. B. Ventral view of the mouth area, scale bar = 1 mm. Abbreviations: a, ampulla; bc, bursa copulatrix; dd, deferent duct; f, female glands; pr, prostate; sr, seminal receptacle; v, vagina.

The background color is reddish cream (Figure 1C). The entire dorsum is covered with a number of short, irregular, very densely arranged dark gray lines. Most of the lines are ramified into irregular branches. There are also irregular white areas, composed of the aggregation of numerous opaque white spots. The tubercles are pale brown or ochre, with the apex darker. A pale brown or ochre irregular line surrounds the mantle margin. The gill and rhinophores are pale brown to cream. The branchial leaves are covered with numerous minute white dots.

The anterior border of the foot is not grooved or notched, with anteriorly directed foot margins partially surrounding the mouth area (Figure 5B). There are no oral tentacles.

**Anatomy:** The jaws are composed of numerous, long undivided rodlets (Figure 6D). The radular formula is  $69 \times 29.0.29$  in the 35 mm-preserved-length specimen (CASIZ 068651). Rachidian teeth are absent. The innermost lateral teeth are broad and elongate (Figure 6A). They have



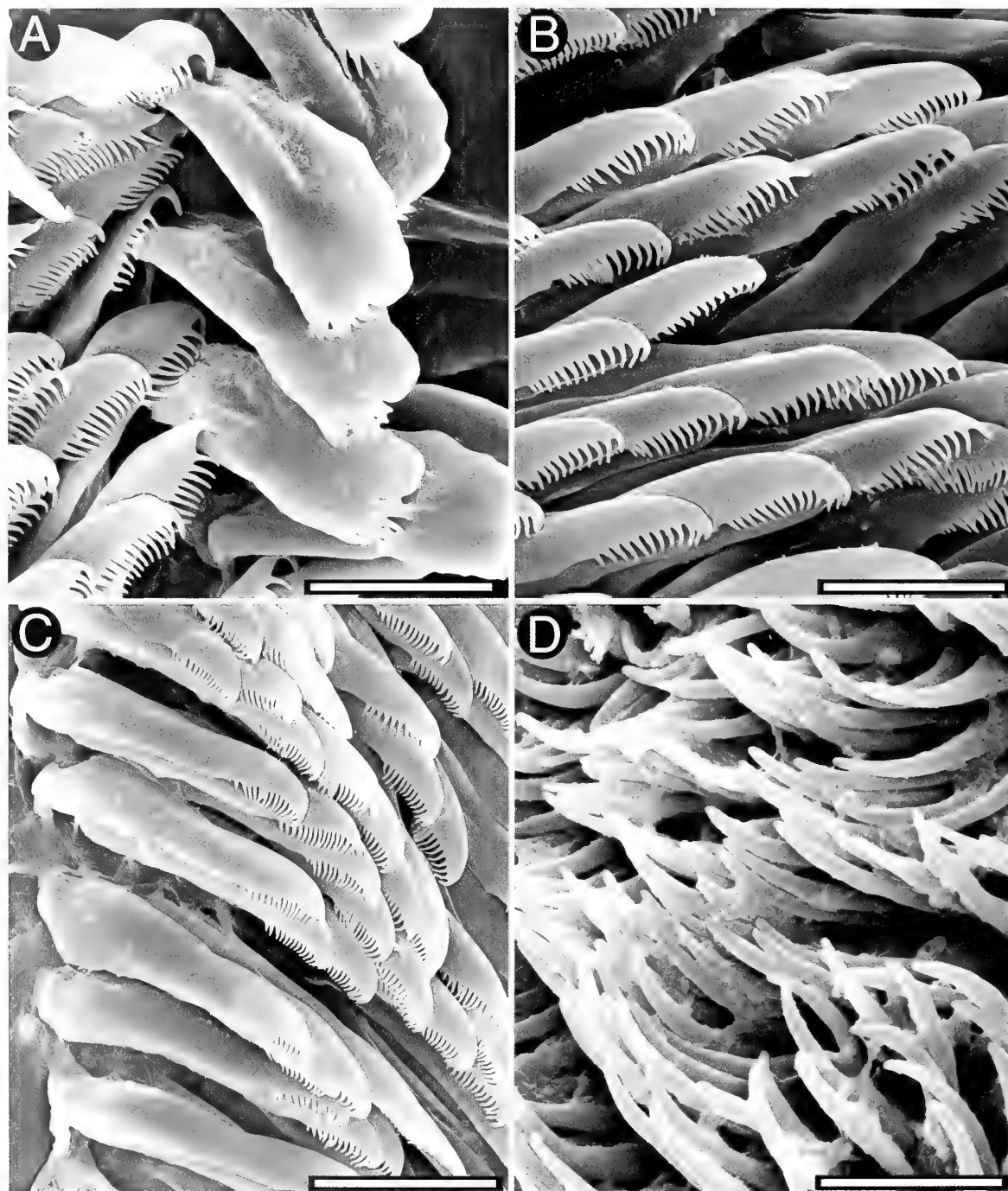


Figure 6. *Actinocyclus papillatus* (CASIZ 068651), scanning electron micrographs. A. Innermost lateral teeth, scale bar = 35  $\mu\text{m}$ . B. Mid-lateral teeth, scale bar = 35  $\mu\text{m}$ . C. Outermost lateral teeth, scale bar = 40  $\mu\text{m}$ . D. Jaw elements, scale bar = 10  $\mu\text{m}$ .

a very short and wide rounded cusp and six to eight elongate denticles along their inner edge. The mid-lateral teeth have a narrow base and are elongated (Figure 6B). They are also multidenticulated, with 12–16 long denticles. The outermost teeth are shorter than the mid-laterals,

but very similar in shape (Figure 6C). The outer laterals have 12–16 denticles along their inner margin.

The narrow hermaphroditic duct expands into the large ampulla, which inserts distally at the junction of the oviduct and the prostate (Figure 5A). The oviduct enters the

female gland in the center of the mass. The prostate is oval, almost as long as wide, and granular. It connects with a long duct that narrows and expands again into the short ejaculatory portion of the deferent duct. The muscular ejaculatory portion opens into a common atrium with the vagina. The penis is unarmed. The vagina is very long and undulate. Near its proximal end it joins the elongate seminal receptacle, the uterine duct, and the rounded bursa copulatrix. The bursa copulatrix is stalked.

**Remarks:** Bergh (1877) first introduced the name *Spaherodoris papillata*, but without a description, and therefore it is a *nomen nudum* as of that paper. Later, Bergh (1878) described the species based on preserved specimens from the Philippines. Examination of the holotype revealed that the dorsum of this species is covered with numerous simple conical to rounded tubercles. The 48 mm-long specimen has 17 unipinnate branchial leaves. The features of this specimen fit with those of the specimen from Papua New Guinea here examined, and they are clearly conspecific.

Eliot (1904) described *Spaherodoris laevis* var. *variegata* based on a single specimen collected from Mnemba Island, Zanzibar, East Africa. The 31 mm-long animal was collected while laying a light violet-colored egg mass and had 14 branchial leaves. The color of the living animal was described as dark brown with greenish and sandy patches. The preserved specimen was mottled brown of darker and lighter shades and had bands formed of minute black spots, arranged in an irregular pattern, particularly near the branchial opening. Eliot (1904) described the dorsum of this species as having "irregular excrescences which resemble a marine growth." The radula had a formula  $70 \times 25.0.25$ . Eliot (1904) compared this animal to other specimens from Malaysia, "apparently referable to *S. laevis* [= *Actinocyclus verrucosus*]," which had the dorsum "quite smooth and of an almost uniform bluish-olive colour." In the same paper, he also mentioned another specimen collected from Mombasa, Kenya, which was different in color, but cannot be identified with certainty from the short description.

Eliot's (1904) description of *Spaherodoris laevis* var. *variegata* fits with the characteristics of the holotype of *Spaherodoris papillata* as well as with those of the specimen from Papua New Guinea. The brownish external color with irregular black lines, the number of branchial leaves, the abundance of large dorsal tubercles, and the radula formula are very similar.

*Actinocyclus papillatus* appears to be a different species from *A. verrucosus*. Externally, *A. papillatus* has more and larger tubercles than *A. verrucosus*. Also it has a number of ramified black lines on the dorsum that are absent in all specimens examined of *A. verrucosus*. Two specimens of *A. papillatus* (32 and 35 mm preserved length) have 14 branchial leaves, and one specimen 48 mm preserved length has 17 (Eliot, 1904; present paper),

whereas smaller specimens of *A. verrucosus* (21 mm preserved length) have 16. A 38 mm-preserved-length specimen of *A. verrucosus* has 21 branchial leaves. Also, the gill of *A. papillatus* is pale brown or cream in color, whereas it is dark gray or black in *A. verrucosus*. In the reproductive system, the seminal receptacle of *A. papillatus* is more elongate than that of *A. verrucosus*. In addition, the ampulla and the prostate seem to be larger in *A. papillatus*. The radula of *A. papillatus* has more lateral teeth than that of *A. verrucosus*, for a specimen smaller in size. Eliot (1904) found a formula of  $70 \times 25.0.25$  for a 31 mm-preserved-length specimen, the formula of the 35 mm-preserved-length specimen from Papua New Guinea is  $69 \times 29.0.29$ , whereas it is  $65 \times 15.0.15$  in a 35 mm-preserved-length specimen of *A. verrucosus*. No variation has been found in the reproductive system of the three specimens of *A. verrucosus* examined.

Baba (1949) redescribed *A. laevis* var. *variegatus* from Japan and regarded it as different from *A. japonicus*. The external morphology of the single specimen was unknown. He found differences in the radular morphology and jaws. According to Baba, the inner radular teeth of *A. laevis* var. *variegatus* are longer and thinner than those of *A. japonicus*. Also, the jaw elements of *A. laevis* var. *variegatus* are simple, whereas those of *A. japonicus* may be simple or bifid. I have found a similar variation within a single specimen of *A. verrucosus*. The younger teeth normally look like those described by Baba (1949) for *A. laevis* var. *variegatus*, whereas the teeth from the middle of the radula are broader with longer denticles. However, the radular formula of Baba's specimen is  $80 \times 25-28.1.0.1.25-28$  (for a 30 mm-preserved-length specimen), which is certainly very similar to formulae of our material of *A. papillatus*. It is very likely that his material actually belongs to *A. papillatus*. Baba (1949) also described several specimens of *A. japonicus*, which have a grayish brown dorsum, boldly variegated with black-brown, and all the dorsal tubercles tipped with black-brown. This description and the radular formula  $120 \times 30.1.0.1.30$  fit with the characteristics of *A. papillatus*, and should probably be assigned to this species.

Hori & Fukuda (1996) described several specimens from Japan, under the name *A. japonicus*, that fit with the external morphology and anatomy of *A. papillatus*. The specimens have a pale background color with a number of dark, irregular, and ramified lines covering the entire dorsum, the gill is light brown or cream, and the radular formula is  $80 \times 22-30.0.22-30$  for a 47 mm-preserved-length specimen.

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## *Owengriffithsius*, a New Genus of Cyclophorid Land Snails Endemic to Northern Madagascar

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**Abstract.** *Owengriffithsius*, gen. nov. is diagnosed anatomically by its bursa copulatrix consisting of two adjacent ductless sacs; and by its bulbous-tipped penis in which the seminal tube is enclosed (no seminal groove), apically looped, and subapically opening, and which bears a thick, semicircular, flaplike gland. It is diagnosed conchologically by its concavely conic high spire with slightly mamillate apex; its V-channeled suture throughout ontogeny; its suturally notched double peristome; its relatively broad umbilicus (umbilical width/shell diameter 0.22–0.29 in known adults); its medium size (9.1–12.2 mm diameter in known adults); its periostracal hairs; its round and nearly planar outer peristome; and its horny, single-layered operculum. The genus contains six species and one subspecies, all of which are new and described herein. A dichotomous key is provided to their identification.

### INTRODUCTION

This paper is one in a series reporting taxonomic results from the author's 1992–1996 survey and inventory of Madagascar's land mollusks (Emberton, 1998, 1999a, b, 2000, 2001, 2002, in press a, b, c, d; Emberton & Pearce, 1999a; 2000a, b, c).

### METHODS AND MATERIALS

Materials were collected 1994–1996 using methods of Emberton et al. (1996). Identification and comparisons were made using Bequaert & Clench (1936), Wenz (1938–1944), Tielecke (1940), Morton (1952), Zilch (1959–1960), Solem (1959), Verdcourt (1963, 1964), Thompson (1969), Girardi (1978), Bruggen (1982, 1985, 1986, 1990), Fischer-Piette et al. (1993), and Emberton & Pearce (1999). Templeton's (1989) cohesion concept was applied in delimiting species. Geographically separated, morphologically discrete, extreme variants were deemed subspecies only if they seemed well isolated by discontinuous habitat.

Measurements were made using an ocular micrometer on a Wild M3C dissecting microscope. Dissections were on black wax under 70% ethanol, using procedures of Emberton & Pearce (1999: figures 32, 49, 50); anatomical descriptive terminology followed Girardi (1978). Photographs were taken at standard magnifications ( $\times 6.4$ ,  $\times 10$ ,  $\times 16$ ,  $\times 25$ , and  $\times 40$ ).

### LOCALITIES

Of the 1126 stations collected throughout Madagascar in 1992–1996, only the following 17 stations—all northern—yielded *Owengriffithsius*, gen. nov.

74. Namoroka Reserve, 16°23'S, 45°20'E, 100 m, dry deciduous forest, 28 May 1995.  
101, 102. Tsaratanana Reserve, 14°02'S, 48°47'E, rain-forest. 101. 1100 m, 15 June 1995. 102. 1000 m, 16 June 1995.  
206–213. Analamera Reserve, dry deciduous forest, 16 July 1995. 206, 208. 12°44'S, 49°30'E. 206. 195 m. 208. 100 m. 213. 12°44'S, 49°29'E, 30 m.  
218. Montagne des Orchides, 12°23'S, 49°19'E, 385 m, dry deciduous forest, 20 July 1995.  
405–407. Cap d'Ambre, Ambongoabo, 12°15'S, 49°15'E, 25 August 1995. 405, 406, baobab-deciduous forest. 405. 320 m. 406. 310 m. 407. 290 m, dry deciduous forest.  
411. W of Sakaramy, S of Diego Suarez, 12°26'S, 49°12'E, 380 m, dry deciduous forest, 26 August 1995.  
569–580, 803–807. Ankarana Reserve & vicinity, dry deciduous forest. 569. 12°56'S, 49°07'E, 130 m, 23 August 1995. 571. 12°57'S, 49°07'E, 85 m, dry deciduous forest, 24 August 1995. 577. 12°58'S, 49°06'E, 100 m, 25 August 1995. 580. 12°58'S, 49°05'E, 95 m, 26 August 1995. 803. 13°00'S, 49°01'E, 50 m, 8 October 1994. 807. 12°54'S, 49°06'E, 90 m, 10 October 1994.

### SYSTEMATICS

Higher classification follows Ponder & Lindberg (1997; above superfamily) and Vaught (1989; superfamily and family). Latitudes and longitudes are given in degrees and minutes. To aid future workers, alcohol-preserved paratypes are listed separately, and species descriptions are ordered alphabetically. Types are placed in the Florida Museum of Natural History, University of Florida,

Gainesville (UF); the Australian Museum, Sydney (AMS); the Academy of Natural Sciences of Philadelphia (ANSP); and the Muséum National d'Histoire Naturelle, Paris (MNHN, which does not assign catalogue numbers to types).

#### Class GASTROPODA

#### Clade CAENOGASTROPODA

#### Clade ARCHITAENIOGLOSSA

#### Superfamily CYCLOPHOROIDEA

#### Family CYCLOPHORIDAE

#### *Owengriffithsius* Emberton, gen. nov.

(Figures 1–33)

**Type species:** *Owengriffithsius capdambrae*, sp. nov.

**Other species and subspecies:** *O. capdambrae ankaranae*, subsp. nov., *O. analamerae*, sp. nov., *O. griffithsi*, sp. nov., *O. namorokae*, sp. nov., *O. orchidae*, sp. nov., and *O. tsaratananae*, sp. nov.

**Diagnosis:** A cyclophorid genus unique anatomically in its combination of a bursa copulatrix consisting of two adjacent ductless sacs, and a greatly swollen seminal receptacle; and unique conchologically in its combination of a concavely conic high spire with slightly mamillate apex; a V-channeled suture throughout ontogeny; and, in adults, a suturally notched double peristome. Other diagnostic characters are its bulbous-tipped penis in which the seminal tube is enclosed (no seminal groove), apically looped, and subapically opening, and which bears a thick, semicircular, flaplike gland; and, conchologically, its relatively broad umbilicus (umbilical width/shell diameter 0.22–0.29 in known adults); medium size (known adults 9.1–12.2 mm diameter); periostracal hairs; evenly round, nearly planar, outer peristome; and horny single-layered operculum.

*Spirostoma* Heude, 1885, may possibly have a two-sacced bursa copulatrix (Tielecke's 1940: figure 11 is difficult to interpret), but has a much thinner seminal receptacle and an extremely different shell morphology.

Another undescribed genus from Madagascar (Emberton, unpublished) has a somewhat similar penial structure, but differs in its one-sacced bursa copulatrix and drastically different shell.

Conchologically, *Craspedotropis* Blanford, 1864, and *Japonia* (*Mylicotrochus*) P. & F. Sarasin, 1899, have concavely conic high spires, periostracal hairs, and broad umbilici, but lack both the sutural notch and the double peristome, and they have much more sharply angulate peripheries and peristomes.

*Leptopoma* Pfeiffer, 1847, is similar in shell size and shape, but has a much narrower umbilicus, only rarely has a double peristome, and lacks the deep sutural notch.

*Laqocheilus* Blanford, 1864, can look similar conchologically, but has much stronger spiral sculpture, a thicker peristome, and only a shallow sutural notch.

*Cyclotus* (*Millotorbis*) Fischer-Piette & Bedoucha, 1965, has a double peristome with sutural notch, a broad umbilicus, and periostracal hairs, but its spire is not concave, its outer peristome is non-circular and non-planar, and its shell size is minute (2.5–3.6 mm diameter).

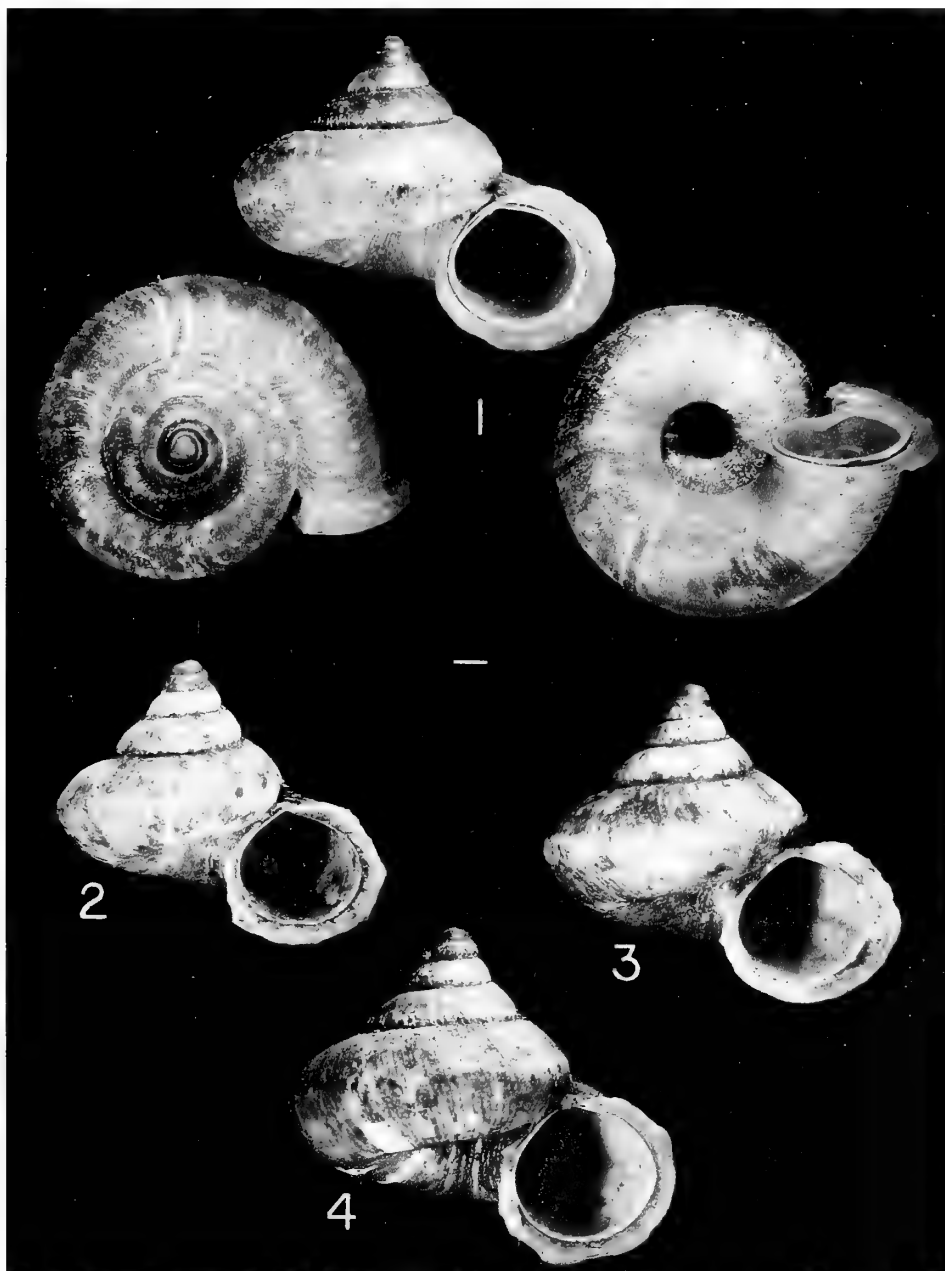
*Cyathopoma* W. & H. Blanford, 1861, is likewise minute, lacks a sutural notch, and has a very different two-layered operculum.

Four species (*Owengriffithsius griffithsi*, sp. nov., *O. namorokae*, sp. nov., *O. orchidae*, sp. nov., and *O. tsaratananae*, sp. nov.) are known only from dead-collected juveniles without opercula. Despite the absence of adult characters, they are clearly distinct, new species, and because they are so rare and unlikely to be collected as adults in the foreseeable future, they are described herein. Their placement in *Owengriffithsius*, gen. nov. is based on the concavely conic high spire with slightly mamillate apex, the V-channeled suture throughout ontogeny, and the round aperture.

Among stylommatophorans (non-operculates), the valoniid (or vertiginid) *Pupisoma* Stoliczka, 1873, is somewhat similar to the latter four species in its aperture, sculpture, and roundish embryonic shell, but its umbilicus is minute, and its size is generally much smaller. If some past *Pupisoma* were to have continued growth with an increased whorl-expansion rate, however, it is conceivable that it evolved into one or more of the four species.

The enid orthurethran *Omphaloconus* (*Omphaloconus*) Westerlund, 1887, is similar in size and shape to *Owengriffithsius griffithsi*, sp. nov., *O. namorokae*, sp. nov., *O. orchidae*, sp. nov., and *O. tsaratananae*, sp. nov., but its aperture is much more elongate and its whorls concomitantly less rounded; its embryonic shell is asymmetrical and less mamillate; and its initial-post-embryonic sculpture seems to differ.

**Description:** Adult shell diameter 9.1–12.2 mm and probably down to about 6 mm, height/diameter of adults and juveniles 0.8–1.3, adult whorls 5.2–6.1, umbilicus/diameter 0.22–0.29 in known adults and down to 0.07 in juveniles. Spire high concave-conic, apex slightly mamillate. Body-whorl periphery angulate, rounded, or bicarinate; suture deeply impressed, a V-shaped channel, beginning at shell's very apex. Aperture round, moderately capacious. Adult peristome double-lipped: inner lip unreflected; outer lip broadly and somewhat flatly reflected, but with a deep sutural notch, and narrow facing the umbilicus. Embryonic whorls 2.4–2.8; first 1.5 whorls 0.85–1.19 mm in diameter. Embryonic sculpture generally initially smooth, then with thin, dense axial riblets; rarely with axially oriented granulae instead. Body-whorl sculpture with axial, periostracal riblets or lamellae, the latter fringed densely with hairlike processes (usually worn off)



Figures 1–4. Dead-collected shells of *Owengriffithsius capdambrae* Emberton, gen. nov., sp. nov. Figure 1. Holotype in three views (UF 285461). Figures 2–4. Paratypes in one view: Figure 2, from type locality, Cap d'Ambre (UF 285460); Figures 3 & 4, from southwest of Diego Suarez (UF 285459, specimens #1 & #2 respectively). Scale bar 1 mm.

that are greatly elongate at the shell's—when present—peripheral angulation, carinae, and/or mid-basal spiral cord; or with minute, fairly evenly and densely spaced, somewhat wavy spiral cordlets.

Operculum fairly thin, horny, yellow with slight orangish tinge, circular, with parietal edge straight and rolled inward. Nucleus central. Opercular whorls gradually and evenly increasing, approximately equal in number to shell

whorls. On both internal and external surfaces, whorls are rimmed with a thin low ridge. On external surface, about the third whorl and all subsequent whorls with extremely thin outer edges broadly overlapping subsequent whorls, producing a layered appearance. On internal surface, outermost whorl smooth and glossy, but all other surfaces somewhat rough and non-reflective.

Foot relatively short and broad, undivided. Snout (with

buccal mass unprotruded) short, divided centrally into two lobes. Protruded buccal mass (with mouth and radula showing) large, broad, rounded, un-notched, with no evident jaw, the two lobes of the snout appearing on the sides of its base like tiny lappets. Body gray, dorsal surfaces of the tentacles darker, snout lighter; mantle orangish cream with dark gray spattering. Testis large, nearly completely displacing apical digestive gland. Penis fairly long, thickly tubular, basally crooked, distally swollen and bulbous. Penial pore subapical, behind distal bulb, opening to side of penis. Ejaculatory duct coursing beneath surface of distal bulb and arcing backward before exiting at pore, and containing a long, muscular, terminal, invaginable, intromittant portion of penis. Within distal bulb of penis, on dorsal side, a white mass of glandular tissue is visible. Left side of penis bearing a smallish, thick, semi-circular, flaplike gland that rolls partially around penial shaft. Ovary lying along inside curve of apical digestive gland and consisting of tightly packed, bulbous acini. Oviduct (= "tube of FPSC" of Emberton & Pearce [1999]) with sharp, V-shaped bend before running alongside, then tapering into, seminal receptacle. Seminal receptacle (= "albumen gland" of Thompson [1969] = "glandular base of FPSC" of Emberton & Pearce [1999]) with slight-to-pronounced S-curve at its (proximal) junction with oviduct, thereafter C-shaped and greatly swollen, but tapering and straightening distally. Bursa copulatrix (= "seminal receptacle" of Thompson [1969] = "gland of FPSC" of Emberton & Pearce [1999]) consisting of two adjacent, ductless sacs, the upper (proximal) larger than the lower (distal).

**Etymology:** For Owen Griffiths of Mauritius, in recognition of his collection of, research on, and sponsoring of research on land snails, especially of Madagascar and the Mascarenes.

**Gender:** Masculine.

#### KEY TO SPECIES AND SUBSPECIES

- 1a. Initial whorl very small (diameter of first 1.5 whorls 0.85–0.87 mm); coiling very tight (whorls/ln diameter 3.52–4.56) ..... 2
- 1b. Initial whorl larger (diameter of first 1.5 whorls 1.03–1.19 mm); coiling looser (whorls/ln diameter 2.49–3.47) ..... 3
- 2a. Whorl periphery evenly rounded; umbilicus broader, 0.13–0.16 shell diameter .... *O. griffithsi*
- 2b. Whorl periphery slightly angular; umbilicus narrower, 0.07–0.11 shell diameter ..... *O. orchidae*
- 3a. Initial embryonic sutural channel granulate and with sutural radial marks; embryonic coiling tighter (diameter of first 1.5 whorls 1.03–1.04); general coiling also tighter (whorls/ln diameter 3.27–3.47) ..... *O. namorokae*
- 3b. Initial embryonic sutural channel smooth and

- without sutural radial marks; embryonic coiling looser (diameter of first 1.5 whorls 1.10–1.19); general coiling also looser (whorls/ln diameter 2.49–2.83) ..... 4
- 4a. Embryonic axial riblets dissected by dense, engraved spiral lines; body-whorl sculpture of minute, dense, parallel spiral cords; whorl periphery round, without angulation or carination ..... *O. tsaratananae*
  - 4b. Embryonic axial riblets simple, not crossed by spiral lines; body-whorl sculpture of one to three large spiral cords crossed by dense, hair-fringed riblets; whorl periphery angulate or bicarinate .. 5
  - 5a. Embryonic whorls 2.6–2.8; tighter coiling (whorls/ln diameter 2.73 ..... *O. analamerae*
  - 5b. Embryonic whorls 2.4–2.5; looser coiling (whorls/ln diameter 2.49–2.65) ..... 6
  - 6a. Whorl periphery with single angulation; four spiral color bands ..... *O. capdambrae capdambrae*
  - 6b. Whorl periphery bicarinate; no spiral color bands ..... *O. capdambrae ankaranae*

#### DESCRIPTIONS OF SPECIES AND SUBSPECIES

Abbreviations: ad adult(s), frags fragments, juv juvenile(s).

*Owengriffithsi* *analamerae* Emberton, sp. nov.

(Figures 27–29)

**Diagnosis:** Most similar to *O. capdambrae*, sp. nov., with which it shares (a) large initial whorl (diameter of first 1.5 whorls 1.10–1.19 mm); (b) loose coiling (whorls/ln diameter 2.49–2.83); (c) initial embryonic sutural channel smooth and without sutural radial marks; (d) embryonic axial riblets simple, not crossed by spiral lines; (e) body-whorl sculpture of one to three large spiral cords crossed by dense, hair-fringed riblets; and (f) whorl periphery angulate or bicarinate. *O. analamerae*, sp. nov. differs from *O. capdambrae*, sp. nov. in its greater embryonic whorl count (2.6–2.8 vs. 2.4–2.5) and in its tighter coiling (whorls/ln diameter 2.73 vs. 2.49–2.65).

**Holotype:** Station 213 (UF 285464, 1 ad).

**Illustrated dry paratype:** Station 206 (UF 285465, 1 ad, 1 juv).

**Other dry paratypes:** Stations 206 (UF 285470, 2 juv); 208 (AMS C.203499, 1 ad); 213 (UF 285471, 1 juv).

**Type locality:** Madagascar, Analamera Reserve, 12°44'S, 49°29'E, 30 m, dry deciduous floodplain forest, 16 July 1995.

**Description of holotype shell (Figure 28; broken outer peristome):** Male. Diameter 9.1 mm, height 8.0 mm, whorls 5.7, umbilicus 2.3 mm. Spire high, concave-conic, apex slightly mamillate. Body-whorl periphery angulate;



Table 1

Shell variation. Abbreviations: # specimen number, ColBnds color bands, D1.5W diameter of first 1.5 whorls, Diam shell diameter, EmW embryonic whorl count, fem female, Ht/D shell height divided by shell diameter, juv juvenile, med medium strength, Species/ss species or subspecies, Um/D umbilicus diameter divided by shell diameter, W/lnH shell whorl count divided by natural logarithm of shell height (= index of coiling tightness), Whls shell whorl count.

Species/ss	Catalog #	#	Sex	Diam	Ht/D	Whls	W/lnH	Um/D	D1.5W	EmW	ColBnds
<i>analamerae</i>	UF 285464	—	male	9.1	0.9	5.7	2.73	0.26	1.13	2.8	4 trace
	UF 285465	—	fem?	10.6	1.0	—	—	0.26	—	—	4 trace
	UF 285465	—	juv	—	—	5.2	—	—	1.13	2.6	4 trace
<i>capdambrae</i>	UF 285461	—	fem	12.2	0.8	6.1	2.65	0.29	1.13	2.5	4 faint
	UF 285460	—	male?	10.2	0.9	5.8	2.65	0.23	1.19	—	4 med
	UF 285423	1	fem	12.0	0.9	6.1	2.58	—	1.16	2.5	4 faint
	UF 285462	2	fem	11.7	0.8	6.0	2.62	0.25	1.15	2.5	4 med
	UF 285459	1	male?	10.9	0.9	6.0	2.60	0.22	1.18	—	4 trace
	UF 285459	2	fem	11.8	0.9	6.0	2.54	0.25	1.16	2.4	4 trace
	UF 285422	—	juv	—	—	4.8	—	—	1.15	2.5	4 med
<i>ankaranae</i>	UF 285463	—	fem	10.9	0.9	5.8	2.50	0.21	1.14	2.5	none
	MNHN	—	male	10.6	0.9	5.7	2.51	0.26	1.10	—	none
	AMS C.20350	0	fem?	11.0	0.9	5.9	2.58	0.24	1.10	2.5	none
	UF 285433	1	male	10.2	0.9	5.5	2.50	0.20	1.15	2.5	none
	UF 285434	2	fem	11.1	1.0	6.0	2.49	—	1.10	2.5	none
<i>griffithsi</i>	UF 285430	—	juv	3.2	1.3	5.0	3.52	0.16	0.86	2.5	none
	UF 285431	1	juv	2.4	1.2	4.5	4.29	0.13	0.85	2.5	none
	UF 285431	2	juv	1.9	1.2	3.6	4.56	0.14	0.87	2.5	none
<i>namorokae</i>	UF 285424	—	juv	3.8	1.1	5.0	3.47	0.08	1.03	2.5	none
	UF 285425	—	juv	3.7	1.2	4.9	3.27	0.11	1.04	2.5	none
<i>orchidae</i>	UF 285428	—	juv	2.8	1.2	4.6	3.87	0.07	0.85	2.5	none
	UF 285429	—	juv	2.3	1.1	4.0	4.35	0.11	0.85	—	none
<i>tsaratananae</i>	UF 285426	—	juv	5.4	1.1	4.9	2.77	0.11	1.13	2.5	none
	UF 285427	—	juv	4.8	1.2	4.9	2.83	0.19	1.13	2.5	none

suture deeply impressed, a V-shaped channel, beginning at shell's very apex; whorl shoulders flattish, gently rounded; earlier whorls with rounded shoulder. Aperture round, wide dorsal indentation; height 3.1 mm, width 3.4 mm; downward deflection moderate, 0.5 whorl. Peristome double-lipped: inner unreflected, outer broadly and somewhat flatly reflected, but with a deep sutural notch. Embryonic whorls 2.8; first 1.5 whorls 1.13 mm in diameter. Embryonic sculpture with first 1.2 whorls smooth, then thin, dense riblets. Body-whorl sculpture: faint, spiral, mid-basal cord; low, dense, fairly regularly spaced, axial riblets, with traces of fringing sharp hairs where uneroded, and traces of long broad hairs at peripheral angulation and in a baso-peripheral band. General color brown; white where periostracum eroded away; apex whitish, subsequent upper whorls dark purple-brown. Color bands four, trace, reddish brown: two above and two below peripheral angulation.

**Shell variation:** See Table 1 and Figures 27, 29.

**Etymology:** For *Analamera* Reserve.

### *Owengriffithsius capdambrae* Emberton, sp. nov.

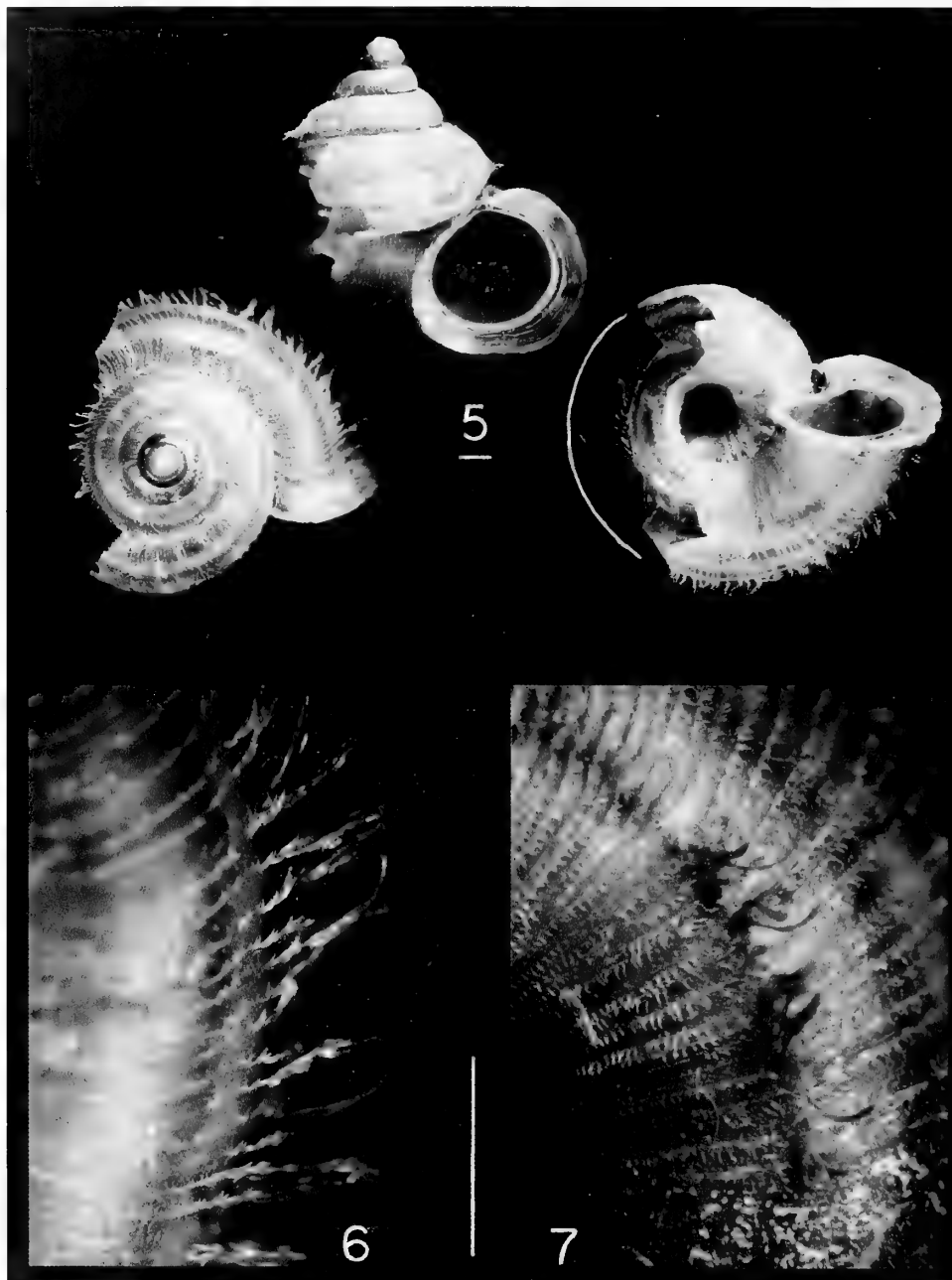
(Figures 1–14)

**Diagnosis:** Most similar to *O. analamerae*, sp. nov., with which it shares (a) large initial whorl (diameter of first 1.5 whorls 1.10–1.19 mm); (b) loose coiling (whorls/ln diameter 2.49–2.83); (c) initial embryonic sutural channel smooth and without sutural radial marks; (d) embryonic axial riblets simple, not crossed by spiral lines; (e) body-whorl sculpture of one to three large spiral cords crossed by dense hair-fringed riblets; and (f) whorl periphery angulate or bicarinate. *O. capdambrae*, sp. nov. differs from *O. analamerae*, sp. nov. in its lesser embryonic whorl count (2.4–2.5 vs. 2.6–2.8) and its looser coiling (whorls/ln diameter 2.49–2.65 vs. 2.73).

**Holotype:** Station 407 (UF 285461, 1 ad).

**Illustrated dry paratypes:** Stations 407 (UF 285462, 1 ad [shell frag and operc from UF 285586]); 411 (UF 285459, 2 ad); 569 (UF 285422, 1 juv).

**Illustrated alcohol paratypes:** Station 407 (UF 285586, 2 ad [dissected]).



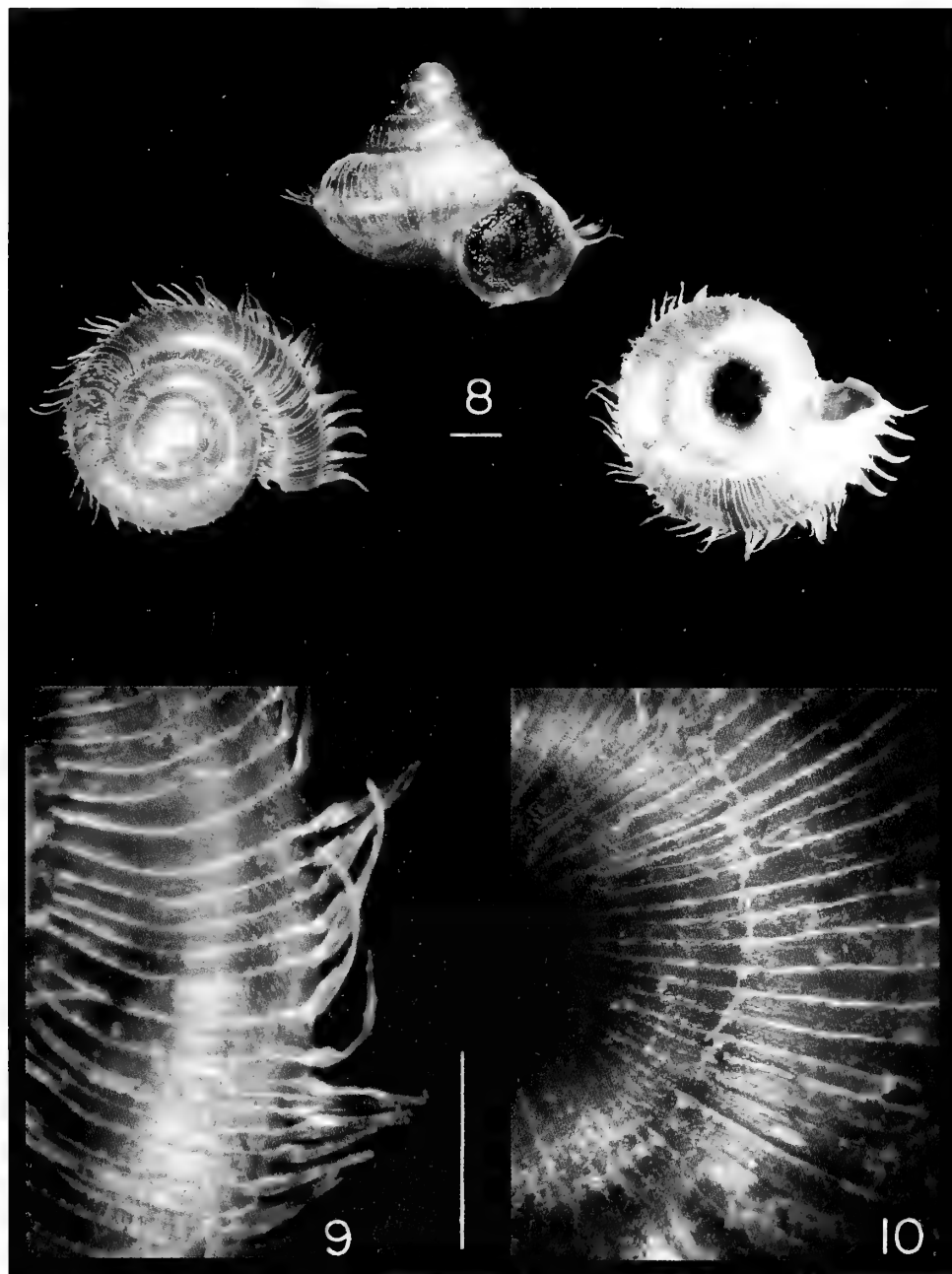
Figures 5–7. Live-collected shell of *Owengriffithsius capdambrae* Emberton, gen. nov., sp. nov., paratype from type locality, Cap d'Ambre (UF 285462). Figure 5. Whole shell (broken) in three views. Figures 6, 7. Magnifications of body whorl from dorsal and ventral aspects, respectively. Scale bars 1 mm.

**Other dry paratypes:** Stations 405 (UF 285584, 3 ad, 3 juv); 406 (UF 285583, 1 ad); 407 (AMS C.203501, 1 ad; ANSP 407928, 1 ad; MNHN, 1 ad; UF 285423, 1 ad [shell frags from UF 285586]; UF 285460, 1 ad [shell frags from UF 285586]; UF 285585, 7 ad, 5 juv); 411 (UF 285582, 2 ad, 3 juv); 571 (UF 285581, 1 juv).

**Type locality:** Madagascar, Cap d'Ambre, Ambongoabo,

12°15'S, 49°15'E, 290 m, dry deciduous forest, 26 August 1995.

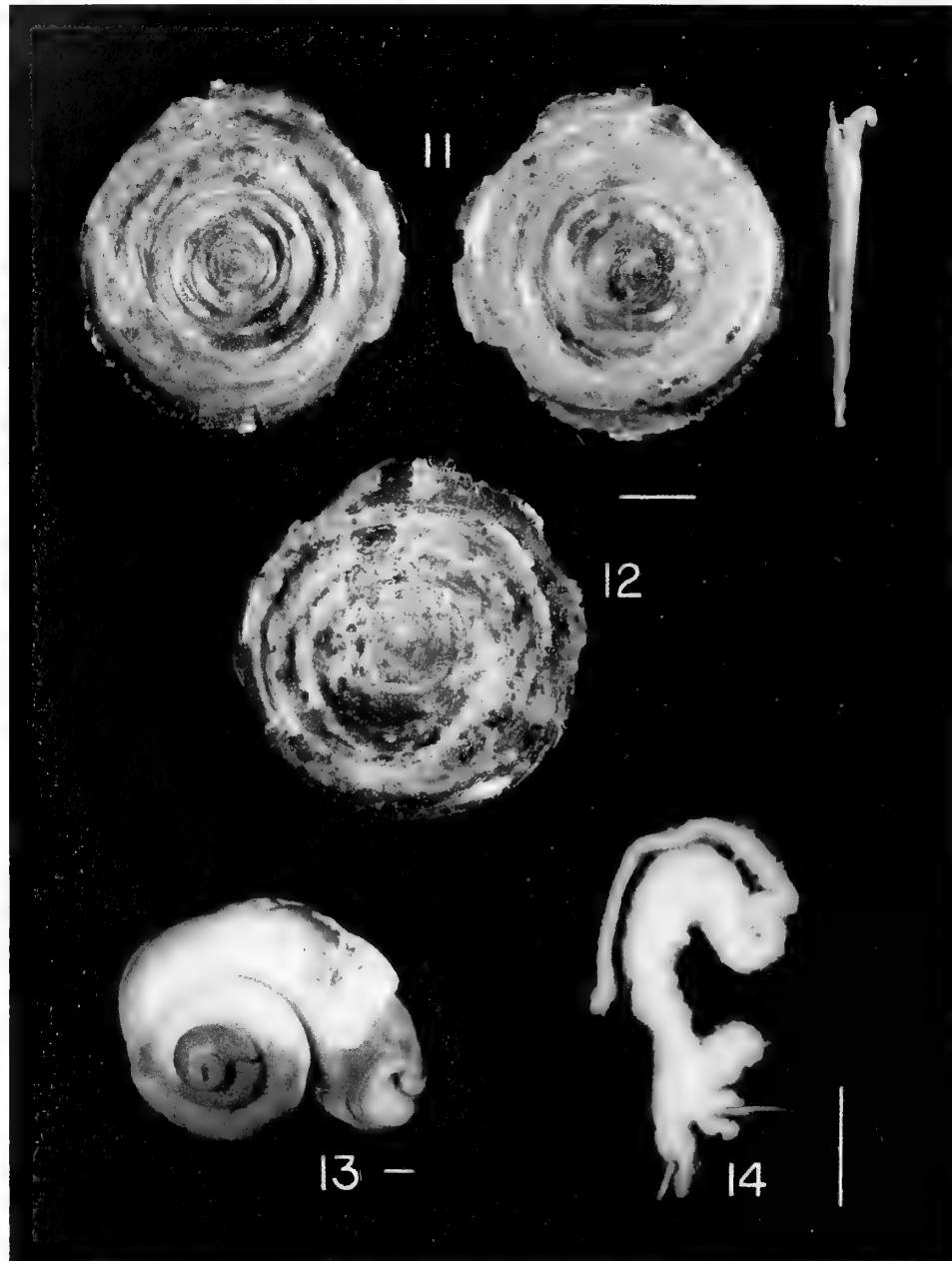
**Description of holotype shell (Figure 1):** Female. Diameter 12.2 mm, height 10.0 mm, whorls 6.1, umbilicus 3.5 mm. Spire high concave-conic, apex slightly mamillate. Body-whorl periphery angulate; suture deeply impressed, a V-shaped channel, beginning at shell's very



Figures 8–10. Fresh-dead-collected juvenile shell of *Owengriffithsius capdambrae* Emberton, gen. nov., sp. nov., paratype from type locality, Ankarana Reserve (UF 285422). Figure 8. Whole shell in three views. Figures 9, 10. Magnifications of body whorl from dorsal and ventral aspects, respectively. Scale bars 1 mm.

apex; whorl shoulders flattish, gently rounded; earlier whorls with rounded shoulder. Aperture round, wide dorsal indentation; height 3.8 mm, width 4.1 mm; downward deflection moderate, 0.3 whorl. Aperture double lipped: inner unreflected, outer broadly and somewhat flatly reflected, but with a deep sutural notch, and narrow facing the umbilicus. Embryonic whorls 2.5; first 1.5 whorls

1.13 mm in diameter. Embryonic sculpture: first 1.4 whorls smooth, then thin, dense riblets. Body-whorl sculpture: faint, spiral, mid-basal cord; low, dense, fairly regularly spaced, axial riblets, with traces of fringing sharp hairs where uneroded behind apertural lip. General color brown; white where periostracum eroded away; apex whitish, subsequent upper whorls dark purple-



Figures 11–14. Opercula and anatomy of *Owengriffithsius capdambrae* Emberton, gen. nov., sp. nov. type-locality females. Figure 11. Operculum of specimen #2 (UF 285462, ex UF 285586) in exterior, interior, and side views (left to right, respectively). Figure 12. Operculum of specimen #1 (UF 285423, ex UF 285586) in exterior view. Figures 13, 14. Body and oviduct-plus-seminal receptacle-plus-bursa copulatrix (removed from the uterus) of specimen #1 (UF 285586). Scale bars 1 mm.

brown. Color bands four, faint, reddish brown: two between suture and peripheral band of hairs, two between peripheral and baso-peripheral bands of hairs.

**Shell variation:** See Table 1 and Figures 2–10. As seen in very fresh shells (Figures 5–10), throughout ontogeny each axial lamella fringed with minute, evenly and densely spaced, sharp-pointed hairs that are uniform in size

except over shell's peripheral angulation and mid-basal spiral cord, where hairs are extremely large and long, forming conspicuous fringing bands around shell. Hairs are readily lost, however, from older weathered shells, and even from shells that are relatively fresh (Figures 1–4).

**Operculum (Figures 11, 12):** Fairly thin, horny, yellow

with slight orangish tinge, circular, with parietal edge straight and rolled inward. Nucleus central. Whorls gradually and evenly increasing, approximately equal in number to the shell whorls. On both the internal and external surfaces, the whorls are rimmed with a thin low ridge. On external surface, about the third whorl and each subsequent whorl with extremely thin outer edge broadly overlapping next whorl, producing layered appearance. On internal surface, outermost whorl smooth and glossy, but all other surfaces somewhat rough and non-reflective.

**Anatomy (Figures 13, 14, ethanol-fixed and -preserved):** Foot relatively short and broad, undivided. Protruded buccal mass (mouth and radula showing) large, broad, rounded, un-notched, with no evident jaw, and with two lobes of snout appearing on sides of its base like tiny lappets. Body gray, dorsal surfaces of tentacles darker, snout lighter; mantle orangish cream with dark gray spattering. Ovary lying along inside curve of apical digestive gland and consisting of tightly packed bulbous acini. Oviduct with a sharp V-shaped bend before running alongside, then tapering into, seminal receptacle. Seminal receptacle with a pronounced S-curve at its (proximal) junction with the oviduct, thereafter C-shaped and greatly swollen, but tapering and straightening distally. Bursa copulatrix consisting of two adjacent, ductless sacs, the upper (proximal) slightly larger than the lower (distal).

**Etymology:** For Cap d'Ambre (Tanjona Bobaomby).

*Owengriffithsius capdambrae ankaranae*  
Emberton, subsp. nov.

(Figures 15–26)

**Diagnosis:** Differs from parent species in its bicarinate (vs. single-angulate) whorl periphery and its lack of spiral color bands (vs. four spiral color bands). Known only from Ankarana Reserve, where it is isolated from parent species by uninhabitable savannah.

**Holotype:** Station 577 (UF 285463, 1 ad).

**Illustrated dry paratypes:** Stations 577 (AMS C.203500, 1 ad; MNHN, 1 ad); 580 (UF 285433, 1 ad, 1 operc [shell frags from UF 285594]; UF 285435, 1 juv).

**Illustrated alcohol paratype:** Station 580 (UF 285594, 2 ad [dissected]).

**Other dry paratypes:** Stations 577 (UF 285592, 1 ad, 7 juv); 580 (AMS C.203502, 1 juv; UF 285434, 1 ad [shell frags from UF 285594]; UF 285593, 14 juv); 803 (ANSP 407929, 1 juv; UF 285591, 17 juv); 807 (MNHN, 1 juv; UF 285590, 15 juv).

**Type locality:** Madagascar, Ankarana Reserve, 12°58'S, 49°06'E, 100 m, dry deciduous forest, 25 August 1995.

**Description of holotype shell (Figure 15; outer peristome broken along columellar edge):** Female. Diameter

10.9 mm, height 10.2 mm, whorls 5.8, umbilicus 2.3 mm. Spire concave-conic, apex slightly mamillate. Body-whorl periphery roundish, bluntly bicarinate; suture deeply impressed, a V-shaped channel, beginning at shell's very apex, filled in part by carinal sculpture; whorl shoulders gently rounded. Aperture round, wide dorsal indentation; height 3.8 mm, width 3.9 mm; downward deflection great, 0.4 whorl. Peristome double lipped: inner unreflected, outer broadly and somewhat flatly reflected, but with a deep, narrow, sutural notch, and narrow facing the umbilicus. Embryonic whorls 2.5; first 1.5 whorls 1.14 mm in diameter. Embryonic sculpture: first 1.2 whorls smooth, then thin riblets. Body-whorl sculpture with three spiral cords, or carina: two peripheral and one mid-basal; crossed by fairly regularly spaced, moderately dense, axial, periostracal lamellae; lamellae are low, erect, with serrated edges, becoming clusters of tall, back-curved spikelets where they cross the spiral cords (Figures 18, 19). General color yellowish light brown; apex light reddish brown.

**Shell variation:** See Table 1 and Figures 16, 17. A fresh juvenile shell (Figures 20, 21) shows sculpture similar to adult holotype, but with carinal hairs much longer.

**Operculum (Figure 22):** As in parent species.

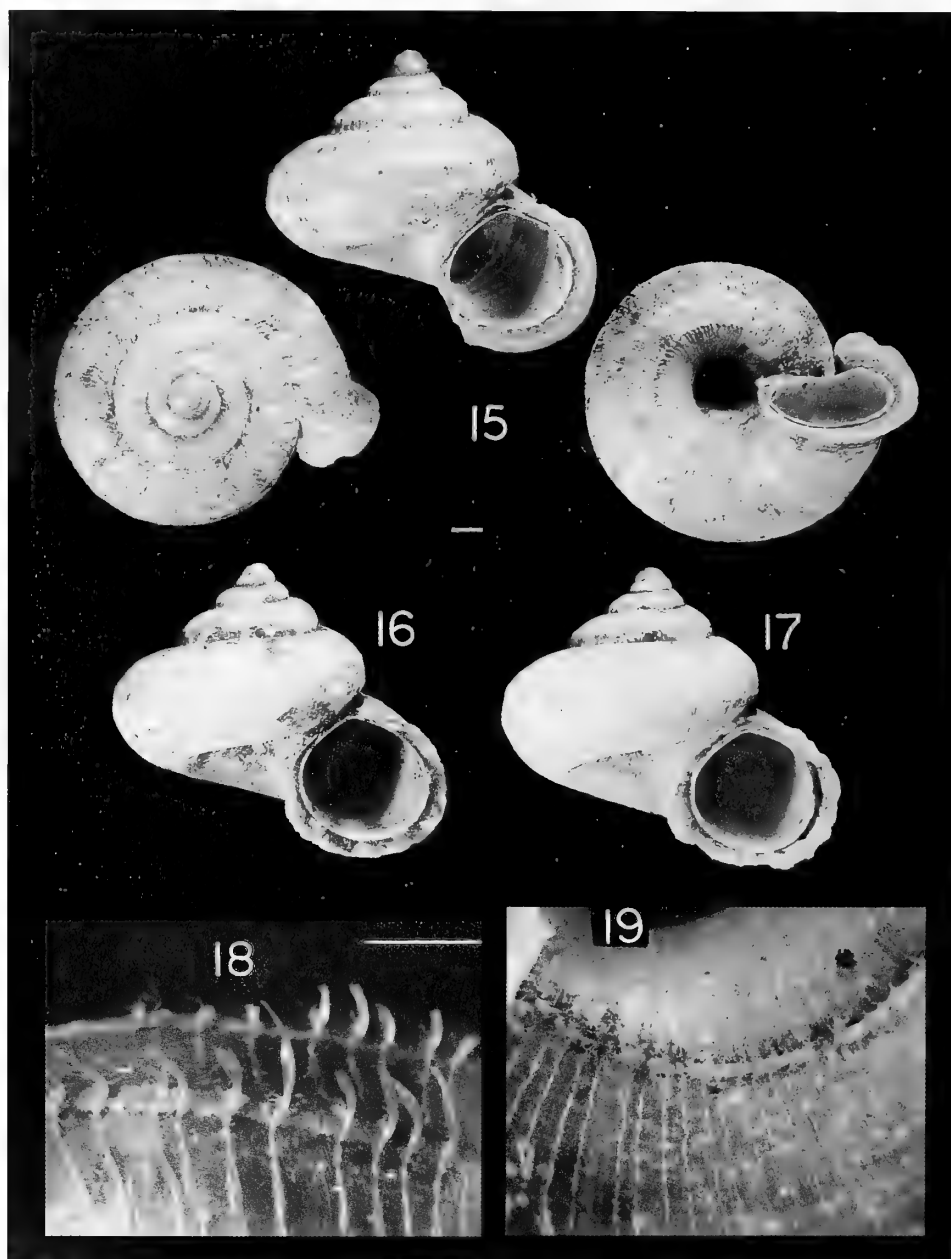
**Anatomy (Figures 23–26, ethanol-fixed and -preserved):** Foot and body as in parent species, but mantle virtually without darker spattering. Snout (with buccal mass unprotruded) short, divided centrally into two lobes. Testis large, nearly completely displacing apical digestive gland. Penis about 4.5 mm, thickly tubular, basally crooked, distally swollen and bulbous. Penial pore subapical, behind distal bulb, opening to side of penis. A longitudinal cross-section of the penis (not illustrated) indicates that ejaculatory duct courses beneath surface of distal bulb, arcs backward before exiting at pore, and contains a long, muscular, terminal, invaginable, intromittant portion of penis. Within distal bulb of penis, on dorsal side, a white mass of glandular tissue visible. Left side of penis bearing a smallish, thick, semicircular, flaplike gland that rolls partially around penial shaft. Female reproductive system as in parent species, except that oviduct and seminal receptacle are less tightly convoluted, and upper (proximal) of bursa-copulatrix sacs much larger than lower (distal).

**Etymology:** For Ankarana Reserve.

*Owengriffithsius griffithsi* Emberton, sp. nov.

(Figure 31)

**Diagnosis:** Most similar to *O. orchidae*, sp. nov., with which it shares a very small initial whorl (diameter of first 1.5 whorls 0.85–0.87 mm) and very tight coiling (whorls/ln diameter 3.52–4.56). *O. griffithsi*, sp. nov. differs from *O. orchidae*, sp. nov. in its evenly rounded (vs.



Figures 15–19. Shells of *Owengriffithsius capdambrae ankaranae* Emberton, gen. nov., sp. nov., subsp. nov. Figure 15. Holotype (dead-collected) in three views (UF 285463). Figures 16, 17. Paratypes (dead collected) from type locality (AMS C.203500 and MNHN respectively). Figures 18, 19. Magnifications of body whorl from dorsal and ventral aspects, respectively, of live-collected paratype (UF 285433). Scale bars 1 mm.

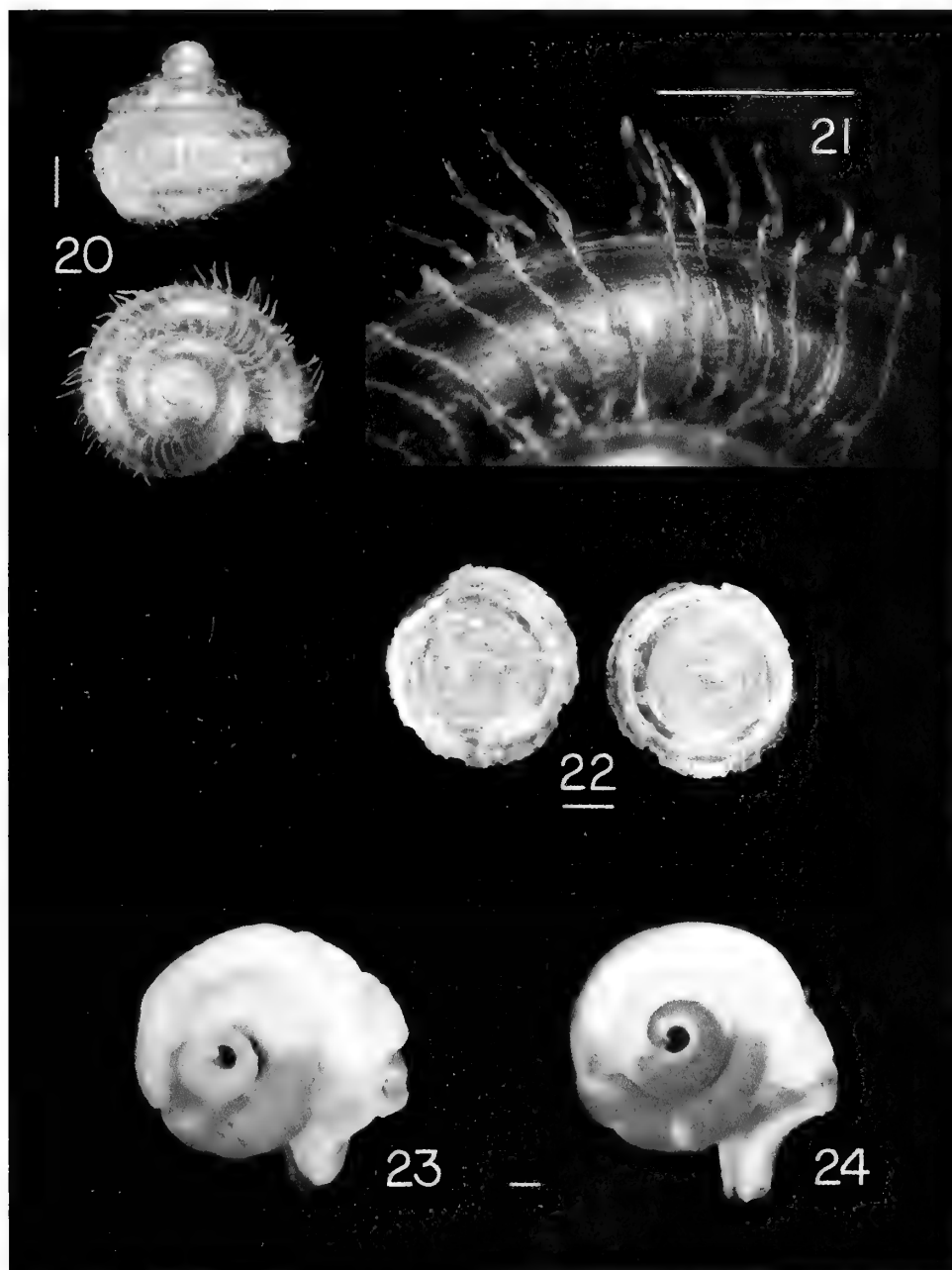
slightly angular) whorl periphery and in its broader umbilicus (0.13–0.16 shell diameter vs. 0.07–0.11 shell diameter).

**Holotype:** Station 803 (UF 285430, 1 juv).

**Dry paratypes:** Stations 803 (AMS C.203503, 2 juv; ANSP 407930, 1 juv; UF 285431, 4 juv); 807 (UF 285432, 1 juv).

**Type locality:** Madagascar, Ankarana Reserve, 13°00'S, 49°01'E, 50 m, 8 October 1994.

**Description of holotype shell (Figure 31; weathered, broken; measurements taken at latest complete aperture):** Juvenile. Diameter 3.2 mm, height 4.1 mm, whorls 5.0, umbilicus 0.5 mm. Spire high concave-conic, apex slightly mamillate. Body-whorl periphery round; suture



Figures 20–24. *Owengriffithsius capdambrae ankaranae* Emberton, gen. nov., sp. nov., subsp. nov. paratypes. Figures 20, 21. Fresh-dead-collected juvenile shell in two views and magnification of dorsal body whorl (UF 285435). Figure 22. Adult operculum in external and internal views (left and right, respectively; UF 285433, ex UF 285594 specimen #2). Figures 23, 24. Adult bodies (UF 285594): Figure 23, male (specimen #1); Figure 24, female (specimen #2). Scale bars 1 mm.

deeply impressed, a V-shaped channel, beginning at shell's very apex; whorl shoulders rounded. Aperture apparently round; height 1.8 mm, width 1.6 mm. Apertural lip unreflected, except slightly at columella. Embryonic whorls 2.5; first 1.5 whorls 0.86 mm in diameter. Embryonic sculpture: initial 1.7 whorls smooth, then densely and regularly spaced, thin-edged low riblets. Body-whorl

sculpture smoothish, with transverse growth striae, uneven in spacing and in strength, but thin-edged and somewhat riblet like. Color uniform grayish white.

**Shell variation:** See Table 1.

**Etymology:** For Owen Griffiths, collector of this species.





Figures 25, 26. Reproductive organs of *Owengriffithsius capdambrae ankaranae* Emberton, gen. nov., sp. nov., subsp. nov. paratypes (UF 285594). Figure 25. Penis in dorsal and ventral views (upper and lower, respectively; specimen #1). Figure 26. Oviduct-plus-seminal receptacle-plus-bursa copulatrix (removed from the uterus; specimen #2). Scale bar 1 mm.

*Owengriffithsius namorokae* Emberton, sp. nov.

(Figure 32)

**Diagnosis:** Among the species of *Owengriffithsius*, gen. nov. having a relatively large initial whorl (diameter of first 1.5 whorls 1.03–1.19 mm), *O. namorokae*, sp. nov. is unique both in its tight coiling (whorls/ln diameter 3.27–3.47 vs. 2.49–2.83) and in having its initial embryonic sutural channel granulate and with sutural radial marks (vs. smooth and without sutural radial marks).

**Holotype:** Station 74 (UF 285424, 1 juv).

**Dry paratype:** Station 74 (UF 285425, 1 juv).

**Type locality:** Madagascar, Namoroka Reserve, 16°23'S, 45°20'E, 100 m, dry deciduous forest, 28 May 1995.

**Description of holotype shell (Figure 32; aperture broken; measurements taken at latest complete aperture):** Juvenile. Diameter 3.8 mm, height 4.2 mm, whorls 5.0, umbilicus 0.3 mm. Spire high concave-conic, apex slightly mamillate. Body-whorl periphery slightly angular; su-

ture deeply impressed, a V-shaped channel, beginning at shell's very apex; whorl shoulders rounded. Aperture apparently round; height 2.0 mm, width 1.9 mm; no downward deflection. Apertural lip unreflected, except slightly at columella. Embryonic whorls 2.5; first 1.5 whorls 1.03 mm in diameter. Embryonic sculpture: initial 1.2 whorls smooth except for sutural channel, which appears granular, thereafter completely granular with traces of axial orientation; initial whorl also with sutural radial marks. Body-whorl sculpture smoothish, with faint, parallel, fairly evenly and densely spaced, somewhat wavy spiral cordlets, and with faint, irregular axial growth striae; cordlets absent on base. Color uniform grayish white.

**Shell variation:** See Table 1.

**Etymology:** For Namoroka Reserve.

*Owengriffithsius orchidae* Emberton sp. nov.

(Figure 33)

**Diagnosis:** Most similar to *O. griffithsi*, sp. nov., with which it shares a very small initial whorl (diameter of first 1.5 whorls 0.85–0.87 mm) and very tight coiling (whorls/ln diameter 3.52–4.56). *O. orchidae*, sp. nov. differs from *O. griffithsi*, sp. nov. in its slightly angular (vs. evenly rounded) whorl periphery and its narrower umbilicus (0.07–0.11 shell diameter vs. 0.13–0.16 shell diameter).

**Holotype:** Station 218 (UF 285428, 1 juv).

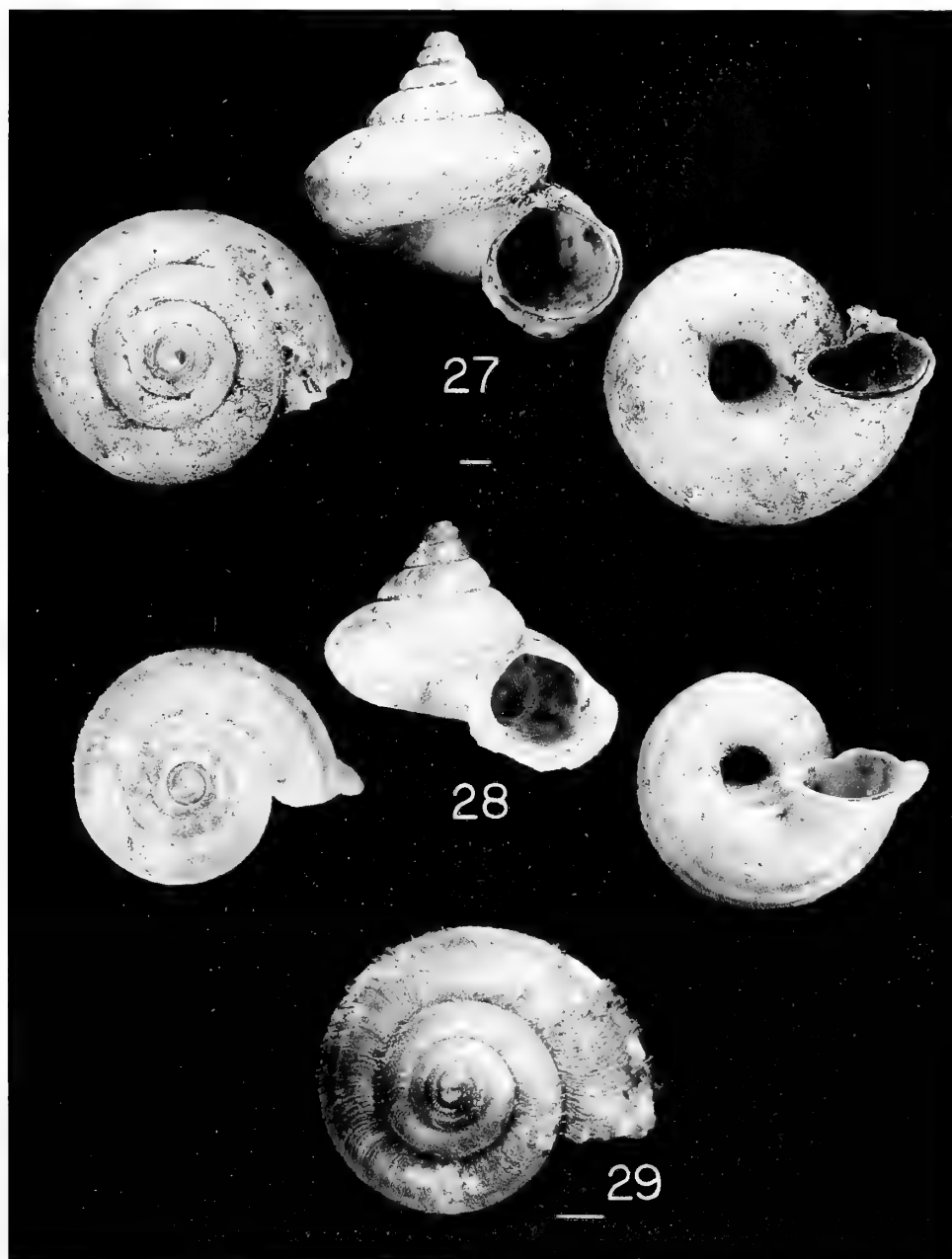
**Dry paratypes:** Station 218 (AMS C.204775, 1 juv; ANSP 407931, 1 juv; MNHN, 1 juv; UF 285429, 2 juv).

**Type locality:** Madagascar, Montagne des Orchides, 12°23'S, 49°19'E, 385 m, dry deciduous forest, 20 July 1995.

**Description of holotype shell (Figure 33; aperture broken; measurements taken at latest complete aperture):** Juvenile. Diameter 2.8 mm, height 3.3 mm, whorls 4.6, umbilicus 0.2 mm. Spire high concave-conic, apex slightly mamillate. Body-whorl periphery slightly angular; suture deeply impressed, a V-shaped channel, beginning at shell's very apex; whorl shoulders rounded. Aperture apparently round; height 1.5 mm, width 1.5 mm; no downward deflection. Apertural lip unreflected, except slightly at columella. Embryonic whorls 2.5; first 1.5 whorls 0.85 mm in diameter. Embryonic sculpture: initial 1.8 whorls smooth, then strong, dense, regularly spaced, sharp-edged riblets. Body-whorl sculpture consisting of low, sharp axial riblets, sparsely and unevenly spaced, uneven in strength. General color light brown, apex whitish.

**Shell variation:** See Table 1.

**Etymology:** For Montagne des Orchides.



Figures 27–29. Shells of *Owengriffithsius analamerae* Emberton, gen. nov., sp. nov. Figure 27. Paratype in three views (UF 285465, specimen #1). Figure 28. Holotype in three views (UF 285464). Figure 29. Magnification of juvenile paratype (UF 285465, specimen #2). Scale bars 1 mm.

*Owengriffithsius tsaratananae* Emberton, sp. nov.

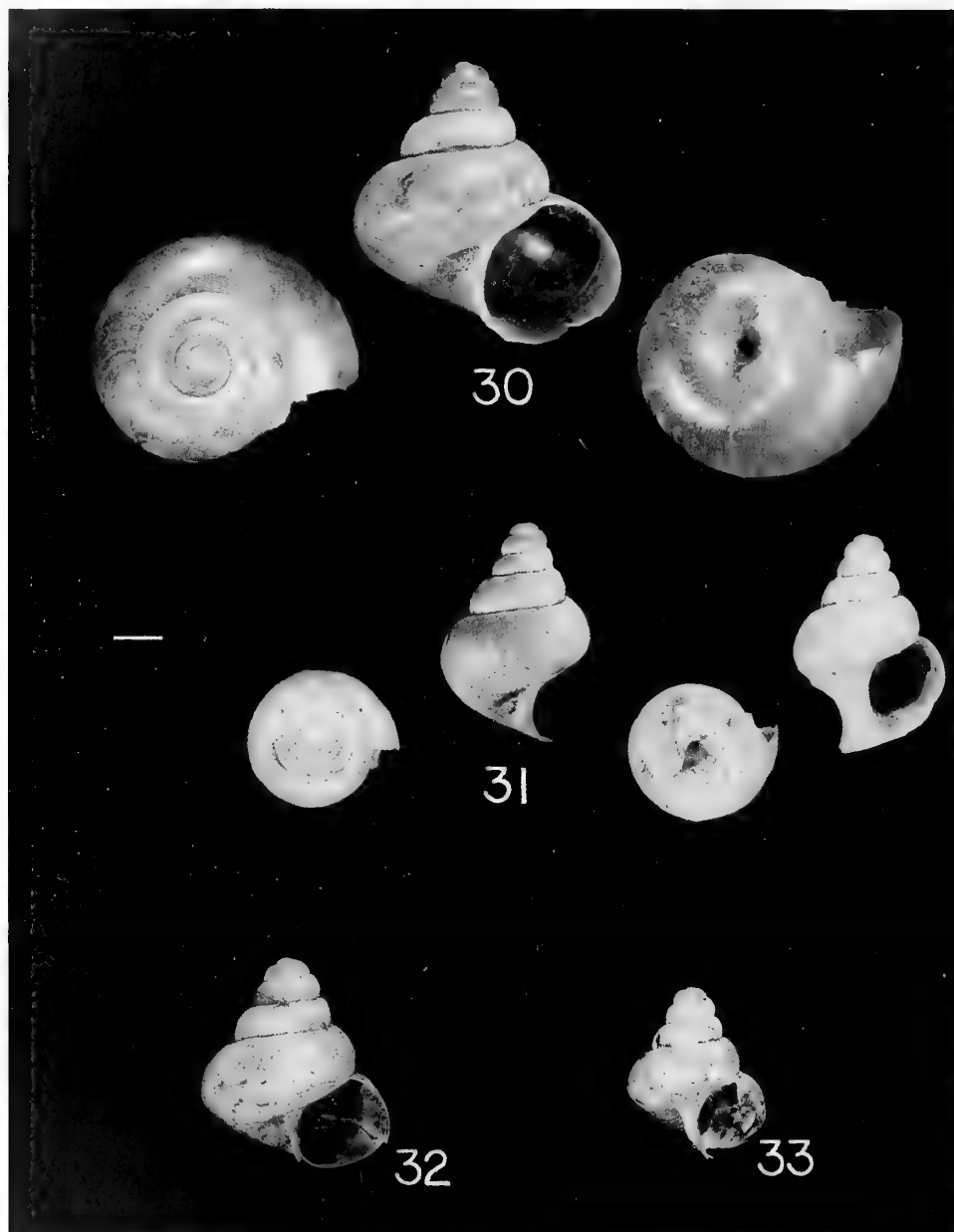
(Figure 30)

**Diagnosis:** Among the species of *Owengriffithsius* gen. nov. having (a) large initial whorl (diameter of first 1.5 whorls 1.10–1.19 mm), (b) loose coiling (whorls/ln diameter 2.49–2.83), and (c) initial embryonic sutural channel smooth and without sutural radial marks, *O. tsaratan-*

*anae*, sp. nov. is unique for its (a) embryonic axial riblets dissected by dense, engraved spiral lines; (b) body-whorl sculpture of minute, dense, parallel spiral cords; and (c) whorl periphery round, without angulation or carination.

**Holotype:** Station 101 (UF 285426, 1 juv).

**Dry paratypes:** Station 102 (AMS C.204504, 1 juv; ANSP 407932, 1 juv; MNHN, 1 juv; UF 285427, 5 juv).



Figures 30–33. Holotype shells of species of *Owengriffithsius* Emberton gen. nov., all to same scale. Figure 30. *Owengriffithsius tsaratananae* Emberton, sp. nov., in three views (UF 285426). Figure 31. *Owengriffithsius griffithsi* Emberton, sp. nov., in four views (UF 285430). Figure 32. *Owengriffithsius namorokae* Emberton, sp. nov. (UF 285424). Figure 33. *Owengriffithsius orchidae* Emberton, sp. nov. (UF 285428). Scale bar 1 mm.

**Type locality:** Madagascar, Tsaratanana Reserve, 14°02'S, 48°47'E, 1100 m, rainforest, 15 June 1995.

**Description of holotype shell (Figure 30; aperture broken; measurements taken at latest complete aperture):** Juvenile. Diameter 5.4 mm, height 5.9 mm, whorls 4.9, umbilicus 0.6 mm. Spire high concave-conic, apex slightly mamillate. Body-whorl periphery round; suture deeply impressed, a V-shaped channel, beginning at shell's very

apex; whorl shoulders rounded. Aperture apparently round; height 3.0 mm, width 2.9 mm. Apertural lip unreflected, except slightly at columella. Embryonic whorls 2.5; first 1.5 whorls 1.13 mm in diameter. Embryonic sculpture: initial 0.5 whorl smooth, then weak dense riblets dissected by dense, engraved spiral lines. Body-whorl sculpture smoothish, with minute, parallel, fairly evenly and densely spaced spiral cordlets separated by narrow

grooves, wavy and/or slightly interrupted where they cross the faint, densely but unevenly spaced, unevenly weak axial growth striae. General color light yellowish brown; apex slightly darker and more reddish.

**Shell variation:** See Table 1.

**Etymology:** For Tsaratanana Reserve.

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## Geographic Variation of Shell Geometry in the Abyssal Snail *Xyloskenea naticiformis* (Jeffreys, 1883)

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**Abstract.** Very little is known about population differentiation in abyssal species. We measured Raup's parameters of shell geometry to document geographic variation in lower bathyal and abyssal populations of the archaeogastropod *Xyloskenea naticiformis* collected in the western North Atlantic. We performed an ANOVA with multiple comparisons to test for interpopulation differences in four basic descriptors of shell form: shape of the generating curve, rate of whorl expansion, position of the generating curve in relation to the coiling axis, and rate of translation. A quantified Jaccard's Coefficient was used to combine the four parameters into a metric of interpopulation phenotypic similarity that was regressed against depth difference and horizontal distance among samples to examine the effects of geographic separation. Overall, geographic variation is muted compared to bathyal deep-sea and coastal marine snails. Significant variation in shell form is largely attributable to a depth difference of 800–1000 m between the lower bathyal zone and the abyssal plain. Abyssal populations show only subtle variation on large (100s km) spatial scales. Results support the theory that population differentiation in mollusks decreases with increasing depth in the deep sea, and that the abyss is less conducive than the bathyal zone to evolutionary divergence in the western North Atlantic.

### INTRODUCTION

It is now well established that the deep sea supports a highly diverse and endemic benthic invertebrate fauna (Hessler & Sanders, 1967; Etter & Mullineaux, 2001), and that community structure varies on local (Grassle & Maciolek, 1992), regional (Etter & Grassle, 1992), and global (Rex et al., 2000) scales. Only recently has attention been devoted to the general problem of how this rich and remarkable fauna evolved (Etter & Rex, 1990; France, 1993; Chase et al., 1998). The primary evidence for understanding patterns of speciation in terrestrial and shallow-water biotas is geographic variation within individual species (Mayr, 1966; Gould & Johnston, 1972; Slatkin, 1987; Palumbi et al., 1997). Documenting and interpreting geographic variation in deep-sea species have proven difficult, particularly at abyssal depths (> 4000 m) where most are very sparsely distributed, and sampling has been limited. In this paper, we analyze geographic variation in shell form of a common and broadly distributed abyssal snail *Xyloskenea naticiformis* (Jeffreys, 1883), collected from the North American Basin of the Atlantic Ocean. Patterns of geographic variation in *X. naticiformis* provide information of basic interest on population differentiation in abyssal species.

### MATERIALS AND METHODS

*Xyloskenea naticiformis* is a minute trochiform archaeogastropod belonging to the family Skeneidae (Warén, 1996). It is widespread at abyssal and bathyal depths of the Atlantic (Warén 1996) and is the second most abundant gastropod species at abyssal depths in the North Atlantic Basin (Rex & Warén, 1982). As with most deep-sea species, little is known of its natural history. The larval shell consists of a single simple whorl measuring 250  $\mu$ m in maximum diameter (Figure 1). This indicates a non-planktotrophic mode of development (Bouchet & Warén, 1994). Whether or not there is a non-feeding pelagic dispersal stage cannot be determined from larval shell morphology in archaeogastropods (Hadfield & Strathmann, 1990). The broad distributions of many deep-sea archaeogastropods and their frequent association with ephemeral patchy habitats (McLean, 1992; Warén & Bouchet, 1993; Warén, 1996; Marshall, 1994) would seem to require dispersal, which in cold bottom currents could involve considerable distances (Hoegh-Guldberg et al., 1991; Shilling & Manahan 1991; Welborn & Manahan, 1991). In every case where a substrate is known, members of the genus *Xyloskenea* in the deep sea are associated with sunken wood (Warén, 1996). There is no record of wood occurring in the samples analyzed here, but this could easily represent a sampling bias. The relatively high abundance and consistent occurrence of *X. naticiformis* in abyssal samples from the western North Atlantic suggest that it might be a facultative deposit feeder as well as grazing on plant debris.

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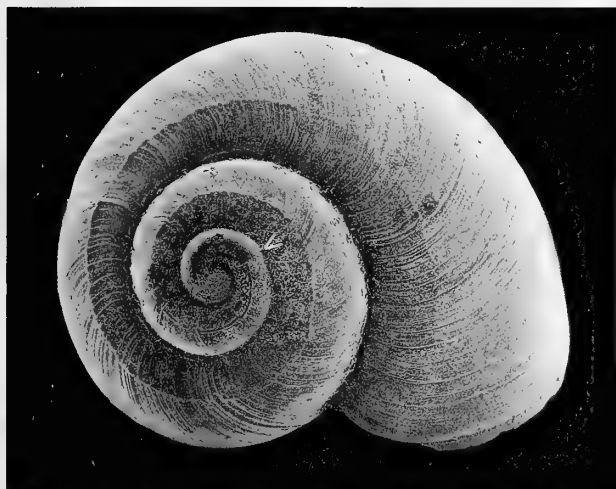


Figure 1. Apical view of a specimen of *Xyloskenia naticiformis* from station 124 (see Table 1 for station data). The arrow indicates the terminus of the larval shell. The larval shell measures 250  $\mu\text{m}$  in maximum width, and the adult shell 1.66 mm in maximum width.

We measured shell form of 152 specimens of *Xyloskenia naticiformis* collected with an epibenthic sled (Hessler & Sanders, 1967) from eight sampling stations in the western North Atlantic (Table 1, Figure 2). One station (85) is located at 3834 m on the lower continental rise, and the other seven are abyssal (4680–4862 m). Our selection criterion was simply to measure all available specimens in sufficiently good condition for samples with  $\geq$  nine such individuals (*X. naticiformis* appeared in seven other samples in this depth range, but with only one to two individuals per sample, which does not permit statistical comparison). The distribution of samples allows us to examine geographic variation in shell form over horizontal scales of up to 100s of kilometers (4–483 km) and bathymetric scales up to about 1000 m (1–1028 m).

In our earlier analyses of geographic variation in deep-sea snails, e.g., Rex et al., 1988; Rex & Etter, 1990 we quantified shell form using an approach developed by Gould (1969) in which shell size, shape, and sculpture are standardized to common growth stages. These standardized measurements were referenced to the terminus of the protoconch which marks the transition from larval to adult growth. These analyses were conducted on risoids and turrids which have relatively high-spired shells so that much of each whorl is exposed for taking measurements. The shells of *Xyloskenia naticiformis* require a different approach. They lack conspicuous sculpture and are much more globular so that early whorls are more obscured by subsequent growth (Figures 1 and 3). Also, in larger specimens, corrosion often makes the adult-larval transition difficult or impossible to discern so that accurate measurements cannot be taken at common growth stages on the adult shell.

However, the simple unadorned shells of *Xyloskenia naticiformis* can be used to estimate Raup's (1966) basic parameters of shell geometry. To measure these parameters we made camera lucida drawings of the shells (at  $\times 50$ ) in two orientations (Figure 3). Following Raup (1966) and Newkirk & Doyle (1975), we approximated the four basic parameters of shell form using the measurements indicated in Figure 3:

- (S) Shape of the Generating Curve. This is expressed as a ratio of the width of the aperture to the height of the aperture.
- (W) Rate of Whorl Expansion. Raup (1966) defined this as:

$$W = \left( \frac{r_2}{r_1} \right)^{2\pi/\theta}$$

where  $r_1$  and  $r_2$  are radii from the axis of coiling to corresponding points on the generating curve (in this case the outer margin of whorls) separated by an angular distance of  $\theta$  radians. We measured radii (OS and OE in Figure 3) every  $45^\circ$ , so the appropriate exponent ( $2\pi/\theta$ ) is eight. Estimates of  $W$  were averaged for each individual (mean = 14, range 8–17 values).

- (D) Position of the Generating Curve in Relation to the Coiling Axis. In most prosobranchs the whorls are wound tightly with the inner margins in contact so that  $D$  is zero. In umbilicate snails like *Xyloskenia naticiformis*,  $D$  is the rate at which whorls move away from the axis of coiling, creating a cone-shaped opening that extends from the bottom of the shell toward the apex.  $D$  is the ratio of the radius at the inner margin of the whorl to that at the outer margin. Again these ratios were measured every  $45^\circ$  and averaged (mean = 15, range 9–18 values).
- (T) Rate of Translation. This is the rate at which whorls move down the axis of coiling in helicoid shells. Raup (1966) defined it as  $dy/dr$ , where  $dy$  is the distance which the center of the generating curve moves down the coiling axis, and  $dr$  is the distance which it moves away from the axis. Since it is difficult to locate the center of the generating curve of successive whorls, we followed Newkirk & Doyle (1975) in approximating  $T$  as the ratio of height of the axis of coiling to the radius (Figure 3).

Averages and standard deviations for  $S$ ,  $W$ ,  $D$ , and  $T$  in all eight populations are given in Table 1.

We performed an ANOVA and Sheffé multiple comparisons among stations for all four variables to test the null hypothesis of no difference in shell form among populations. To get a more general composite picture of interpopulation differentiation, we combined all four parameters of shell geometry into a single measure of phenotypic similarity by using a quantified Jaccard's Coefficient (Sepkoski, 1974) calculated on the average values

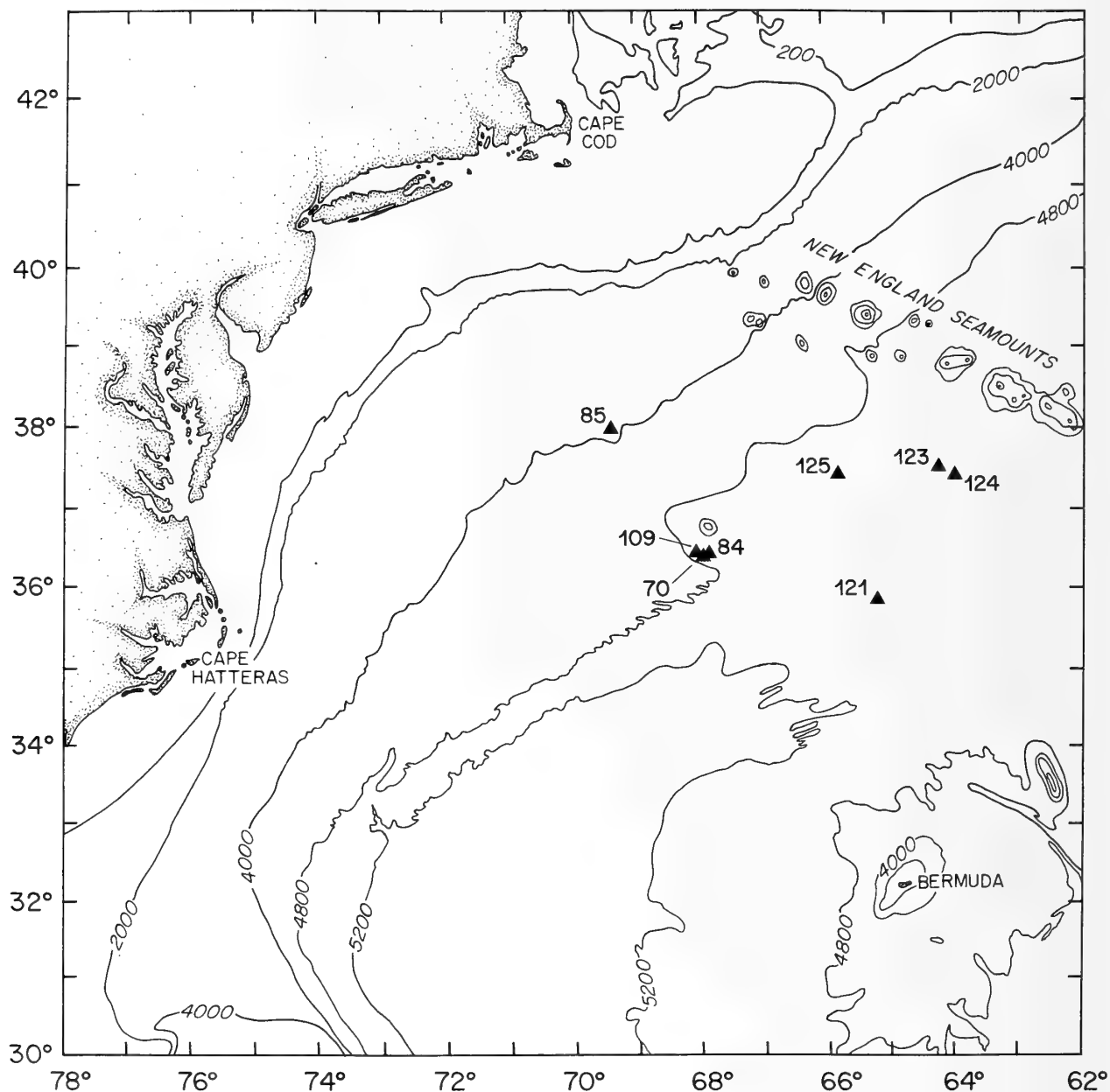


Figure 2. Map of deep-sea benthic stations where samples of *Xyloskenia naticiformis* analyzed in this study were collected. See Table 1 for station data.

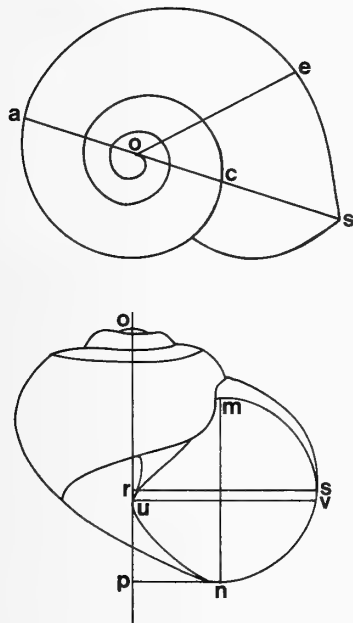
shown in Table 1. To assess whether the degree of phenotypic difference among populations corresponds to the degree of geographic separation, we carried out a multiple regression with Jaccard's Coefficient as the response variable, and depth difference and horizontal distance apart as explanatory variables.

#### RESULTS AND DISCUSSION

Mean values for the four variables of shell form are presented in Table 1. Shells from all localities have very

similar generating curves (width to height ratios of apertures are close to one, Table 1). However, shells from station 85 (at 3834 m) stand out as being uniquely different from all abyssal populations in having a more globular shape: translation rates are lower and whorl expansion rates are higher. Values of *D* are also lower in station 85, i.e., the umbilicus is relatively less well developed because of the higher rate of whorl expansion. The ANOVA reveals a weak overall difference in *S*, and highly significant differences among samples for the other three





$S = (\overline{uv}/\overline{mn})$     $W = (\overline{os}/\overline{oe})^8$     $D = (\overline{oc}/\overline{os})$     $T = (\overline{or}/\overline{os})$   
height =  $\overline{op}$    width =  $\overline{aos}$     $\angle eos = 45^\circ$

Figure 3. Apical and apertural views of *Xyloskenia naticiformis* showing the measurements taken to estimate Raup's (1966) four basic parameters of shell geometry. See text for an explanation of the variables and how shells were measured.

variables (Table 2). Multiple comparison tests show that these differences are largely attributable to station 85 which differs from selected abyssal stations in the same directions noted above (Table 2).

Phenotypic similarity measured as the quantified Jaccard's Coefficient is significantly and negatively correlated with depth differences among samples ( $r = -0.44$ ,  $F_{1,26} = 6.283$ ,  $P = 0.019$ ) due to the distinctiveness of the population from station 85 and the large depth sepa-

Table 2

ANOVA with multiple comparisons for Raup's (1966) four basic parameters of shell geometry (S, W, D, T) for populations of *Xyloskenia naticiformis* in the western North Atlantic. See Table 1 for station data. The inequality signs for the multiple comparison tests indicate a significant ( $P < 0.05$ ) difference and the direction of the difference.

Variable	df	F	Significance	Multiple comparison
S	7,144	2.210	$P = 0.0367$	
W	7,144	3.524	$P = 0.0016$	85 > 121
D	7,144	3.892	$P = 0.0006$	85 < 84
T	7,144	3.489	$P = 0.0017$	85 < 123

ration (~800–1000 m) between station 85 and abyssal stations. The degree of phenotypic similarity is unrelated to horizontal separation ( $F_{1,26} = 3.785$ ,  $P = 0.063$ ). A multiple regression with both distance and depth included as explanatory variables is marginally significant ( $F_{(2,25)} = 3.56$ ,  $P = 0.044$ ).

When the ANOVA is performed on only abyssal stations, differentiation among samples is still detectable, but at a lower level of statistical significance. Translation rate no longer varies significantly ( $F_{6,125} = 1.925$ ,  $P = 0.082$ ). The parameters S, W, and D show significant variation among samples ( $P = 0.028$ , 0.008, and 0.017, respectively) with multiple comparison tests detecting a difference in only one case (for W, 121 < 124). For abyssal samples, the degree of phenotypic similarity is not related significantly to either depth difference ( $F_{1,19} = 0.424$ , n.s.), or distance apart ( $F_{1,19} = 0.433$ , n.s.). A multiple regression analysis using both depth difference and distance is not significant ( $F_{2,18} = 1.518$ , n.s.).

In summary, the clearest divergence in shell form is associated with the large bathymetric difference (800–1000 m) between station 85 on the continental rise and

Table 1

Station data, sample size (n), and the means ( $\bar{x}$ ) and standard deviations (SD) of Raup's (1966) four basic parameters of shell geometry (S, W, D, T) for populations of *Xyloskenia naticiformis* examined in this study. See Figure 2 for a map of station localities. See the text and Figure 3 for a description of the variables and their measurement.

Station	Depth (m)	Latitude (N)	Longitude (W)	n	S		W		D		T	
					$\bar{x}$	(SD)	$\bar{x}$	(SD)	$\bar{x}$	(SD)	$\bar{x}$	(SD)
85	3834	37°59.2'	69°26.2'	20	0.985	(0.048)	2.285	(0.221)	0.416	(0.032)	0.794	(0.085)
70	4680	36°23.1'	67°58.0'	14	0.976	(0.076)	2.210	(0.107)	0.434	(0.013)	0.833	(0.062)
84	4749	36°24.4'	67°56.0'	30	0.971	(0.059)	2.160	(0.138)	0.442	(0.016)	0.869	(0.078)
109	4750	36°25.0'	68°06.0'	9	1.001	(0.089)	2.158	(0.053)	0.424	(0.017)	0.876	(0.074)
121	4800	35°50.0'	65°11.0'	12	0.963	(0.071)	2.053	(0.102)	0.438	(0.015)	0.838	(0.130)
125	4825	37°25.0'	65°52.0'	35	0.978	(0.044)	2.190	(0.160)	0.430	(0.018)	0.861	(0.063)
123	4853	37°29.0'	64°14.0'	15	1.025	(0.056)	2.173	(0.106)	0.429	(0.011)	0.912	(0.066)
124	4862	37°25.0'	63°58.0'	17	1.014	(0.062)	2.244	(0.095)	0.439	(0.017)	0.839	(0.073)

the abyssal stations. The degree of horizontal separation seems unrelated to phenotypic divergence. A lesser degree of differentiation occurs among abyssal samples, and it does not appear to correspond in any consistent way to either depth or horizontal separation. Overall, the geographic variation in shell geometry observed is too idiosyncratic and subtle to warrant speculation about its potential adaptive significance. Also, it is important to recognize that we cannot determine the degree to which it represents selection or phenotypic plasticity (Trussell, 1996). The most significant finding is that *X. naticiformis* shows only very modest geographic variation, particularly among abyssal populations, on quite large geographic scales.

Both the relative degree of geographic differentiation in *Xyloskenea naticiformis* and its association with depth rather than horizontal separation accord well with the overall trend in geographic variation observed in other deep-sea prosobranchs of the western North Atlantic (Rex et al., 1988; Rex & Etter, 1990; Etter & Rex, 1990). Intraspecific differentiation in shell form measured as Mahalanobis' generalized distance ( $D^2$ ) is highest on the upper continental slope and decreases with increasing depth to the abyssal plain (Etter & Rex, 1990). Size-depth clines in gastropod shells also become less pronounced with increasing depth (Rex & Etter, 1998).

Geographic variation in deep-sea species appears to be less well developed than coastal snails show, even on much smaller geographic scales—though most studies of form in shallow-water species focus on a single taxon, the littorinids (cf., e.g., Newkirk & Doyle, 1975; Johannesson 1986; Grahame et al., 1990). Within the deep sea, differentiation tends to be more associated with depth than with horizontal separation (Rex et al., 1988; Rex & Etter, 1990; France & Kocher, 1996). The rate of faunal replacement with depth in snails decreases with increasing depth (Rex, 1977, 1981), and is highly correlated with phenotypic divergence among samples (Etter & Rex, 1990). Both phenotypic change within species and the rate of species turnover reflect the steepness of the environmental gradient which appears to parallel the depth gradient, at least for prosobranchs in the deep western North Atlantic. In contrast to the bathyal environment, the abyssal plain seems to be characterized by a monotonous assemblage of snails and little intraspecific geographic variation in shell architecture on regional spatial scales. Findings presented here for *Xyloskenea naticiformis* support the theory that the abyss is less conducive than the bathyal zone to population differentiation in gastropods of the western North Atlantic (Etter & Rex, 1990; Rex & Etter, 1998).

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## Intermating Interval and Number of Sperm Delivered in the Simultaneously Hermaphroditic Land Snail *Arianta arbustorum* (Pulmonata: Helicidae)

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**Abstract.** The number of sperm delivered is an important determinant for achieving fertilization in sperm competition. Hermaphroditic gastropods with short mating intervals may deplete their autosperm reserves. An earlier study showed that individuals of the simultaneously hermaphroditic land snail *Arianta arbustorum* need at least 8 days to replenish their autosperm reserves after a successful copulation, and that the number of sperm transferred in the second copulation slightly increased up to an intermating interval of 4 weeks. We compared spermatophore size, number of sperm delivered, and mating behavior in snails with longer intermating intervals. Snails that remated after 7–8 weeks did not differ in spermatophore size and number of sperm transferred from individuals that remated after 3–4 weeks. The number of sperm delivered averaged 2,151,000 in the first copulation and 2,130,000 in the second copulation. Snails with a longer intermating interval showed a shorter courtship, but did not differ in copulation duration from snails which remated after 3–4 weeks. Furthermore, different intermating intervals did not affect female fecundity (number of eggs produced and hatching success of eggs). These results indicate that *A. arbustorum* entirely replenishes its autosperm reserves within 3–4 weeks after a successful copulation.

### INTRODUCTION

Pulmonate land snails are simultaneous hermaphrodites with internal fertilization. Individuals of a variety of species mate with two or more different partners in the course of a reproductive season and store foreign sperm for long periods. Promiscuous mating and sperm storage are a prerequisite for sperm competition, i.e., the competition between spermatozoa from two or more males to fertilize the eggs of a single female (Parker, 1970). Sperm competition might significantly affect the reproductive biology of pulmonate land snails. However, with a few exceptions, evolutionary and behavioral aspects of sperm competition have not been examined in terrestrial gastropods (Baur, 1998).

Multiple mating is common in helcid snails. Individuals of *Helix pomatia* (Linnaeus, 1758), *Cepaea nemoralis* (Linnaeus, 1758), and *Arianta arbustorum* (Linnaeus, 1758) have been observed to mate repeatedly with different partners in the course of a reproductive season, resulting in multiple-sired broods (Wolda, 1963; Murray, 1964; Baur, 1988). *Helix pomatia* copulated two to six times per year in a Danish population (Lind, 1988), two to four times in a German population (Tischler, 1973), and *H. aspersa* on average three times (maximum seven times) in a British population (Fearnley, 1993, 1996). Paternity analysis in egg batches of *A. arbustorum* indicated

that at least 63% of the snails used sperm from two or more mates for the fertilization of their eggs (Baur, 1994).

The few data available on mating frequency in gastropods suggest that terrestrial gastropods copulate less frequently than freshwater and marine gastropods (Baur, 1998). In intertidal and terrestrial gastropods, the reproductive activity is limited by favorable environmental conditions (the high risk of desiccation may incur a significant cost of mating). Other explanations for the relatively small number of copulations in terrestrial pulmonates include the cost of mucus production during mating, spermatophore production (in some species), as well as the large number of sperm delivered during a copulation which may result in sperm depletion. A previous study showed that individuals of *A. arbustorum* needed at least 8 days to replenish their sperm reserves after a successful copulation (Locher & Baur, 1999). Furthermore, the number of sperm delivered in the second copulation increased with an increasing intermating interval from 6 to 29 days. This finding suggests that the number of sperm delivered increases with even longer intermating interval. The present study examines this idea.

*Arianta arbustorum* is a simultaneously hermaphroditic land snail common in moist habitats of northwestern and central Europe. The snail has determinate growth (shell breadth of adults 17–22 mm); individuals become sexually mature at an age of 2–4 years, and adults live another 3–4 years (maximum 14 years; Baur & Raboud, 1988). In the field, snails deposit one to three egg batches consisting of 20–50 eggs, per reproductive season (Baur &

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Raboud, 1988; Baur, 1990). Breeding experiments showed that 12 of 44 virgin individuals (27%) prevented from mating produced a few hatchlings by self-fertilization in the second and third years of isolation (Chen, 1993). The reproductive success of selfing individuals, however, was less than 2% of that of mated snails, suggesting high costs for selfing (Chen, 1994).

Mating behavior in *A. arbustorum* includes elaborate courtship behavior with optional dart shooting, i.e., the pushing of a calcareous dart into the mating partner's body), and lasts 2–18 hr (Hofmann, 1923; Baur, 1992a). Copulation is reciprocal; after intromission each snail transfers simultaneously one spermatophore (Haase & Baur, 1995). The spermatophore is formed and filled with sperm during copulation (Hofmann, 1923). It has a distinctive form consisting of a head, a body (sperm container with 800,000–4,000,000 spermatozoa) and a tail 2–3 cm long (Baur et al., 1998; Locher & Baur, 2000). The snails mate repeatedly in the course of a reproductive season, and fertile sperm can be stored for more than 1 year (Baur, 1988). Mating was found to be random with respect to shell size and different degrees of relatedness (Baur, 1992a; Baur & Baur, 1997). A controlled laboratory experiment showed that one successful copulation per reproductive season is sufficient to fertilize all the eggs produced by an individual (Chen & Baur, 1993). However, there is a probability of 5–8% that a copulation will not lead to fertilization of eggs (no sperm transfer or transfer of unfertile sperm; Chen & Baur, 1993).

In the present study we examined whether *A. arbustorum* that remated after 7–8 weeks delivered more sperm than snails remating after 3–4 weeks. We also investigated whether sperm delivery is influenced by courtship and mating behavior in this snail species.

## MATERIALS AND METHODS

### Maintenance of Test Snails

To obtain virgin *A. arbustorum*, subadult individuals that had not yet completed shell growth were collected in a subalpine forest near Gurnigelbad, 30 km south of Bern, Switzerland (46°45'N, 7°27'E; at an elevation of 1320 m above sea level) on 13 May 1999. The snails were kept isolated in transparent plastic beakers (8 cm deep, 6.5 cm in diameter) lined with moist soil mixed with powdered limestone (approximately 4 cm) at  $19^{\circ} \pm 1^{\circ}\text{C}$  and with a light/dark cycle of 16:8 hr for 7 weeks. During this period, subadult individuals reached sexual maturity as indicated by the formation of a flanged lip at the shell aperture. Fresh lettuce was provided *ad libitum* as food. The beakers were cleaned twice per week.

### Mating Trials

Mating trials were performed outdoors to expose snails to natural temperature and light conditions. Two random-

ly chosen active snails (individuals with an extended soft body and everted tentacles) were allowed to copulate in a transparent plastic container, measuring  $14 \times 10 \times 7$  cm, whose bottom was covered with moistened paper towels to maintain activity. One of the two snails was marked on its shell with a spot of correction fluid (Tipp-Ex®) to be able to distinguish between the two partners when recording their behavior. The animals showed no visible reaction to the marking procedure. Mating trials were initiated in the late evening (after 10 p.m.) and ran during 14 nights in June (first copulations) and July and August 1999 (second copulations). The period between the end of May and the middle of July is the time of maximum mating activity in subalpine populations of *A. arbustorum*.

The snails' mating behavior was observed at intervals of 30 min (at night using a flashlight) following the method described in Baur (1992a) and Baur et al. (1998). Records included time until initiation of courtship, courtship duration (time interval from courtship initiation to copulation), and copulation duration. The initiation of courtship was defined as the first simultaneous oral contact, which was usually accompanied by a slight eversion of the penial lobe in one of the snails. The beginning of copulation was defined as the first simultaneous penis intromission. Observation sessions were terminated either when two snails copulated or after 6 hr if no snail initiated courtship behavior in a test arena. Snails that did not mate were tested again 3–7 days later with a new partner. In the period between two trials, the snails were kept isolated as described above. In all, 39 copulations were observed in 177 trials (22.0% successful trials).

After copulation, one mating partner (hereafter referred to as sperm donor) was kept isolated in a transparent plastic beaker lined with moist soil (as described above). The other mating partner (hereafter referred to as sperm recipient) was frozen immediately after copulation.

To assess the influence of the interval between two copulations on the number of sperm delivered, sperm donors were allowed to remate with a randomly assigned virgin partner either 3–4 weeks or 7–8 weeks after the first copulation (Figure 1). One sperm donor of the first group died before the second mating. Seven sperm donors remated after 3–4 weeks (7 copulations in 99 trials; 7.1% successful trials) and 11 sperm donors after 7–8 weeks (11 copulations in 107 trials; 10.3%). In the latter group, two snails did not deliver any spermatophore in the second copulation. These animals were omitted in the data analyses, reducing the sample size of this group to nine.

To assess any size effect of the sperm donor on the number of sperm transferred and number of eggs produced, we measured the size (shell breadth and height) of each mating snail to the nearest 0.1 mm using vernier calipers and calculated the shell volume using the formula:

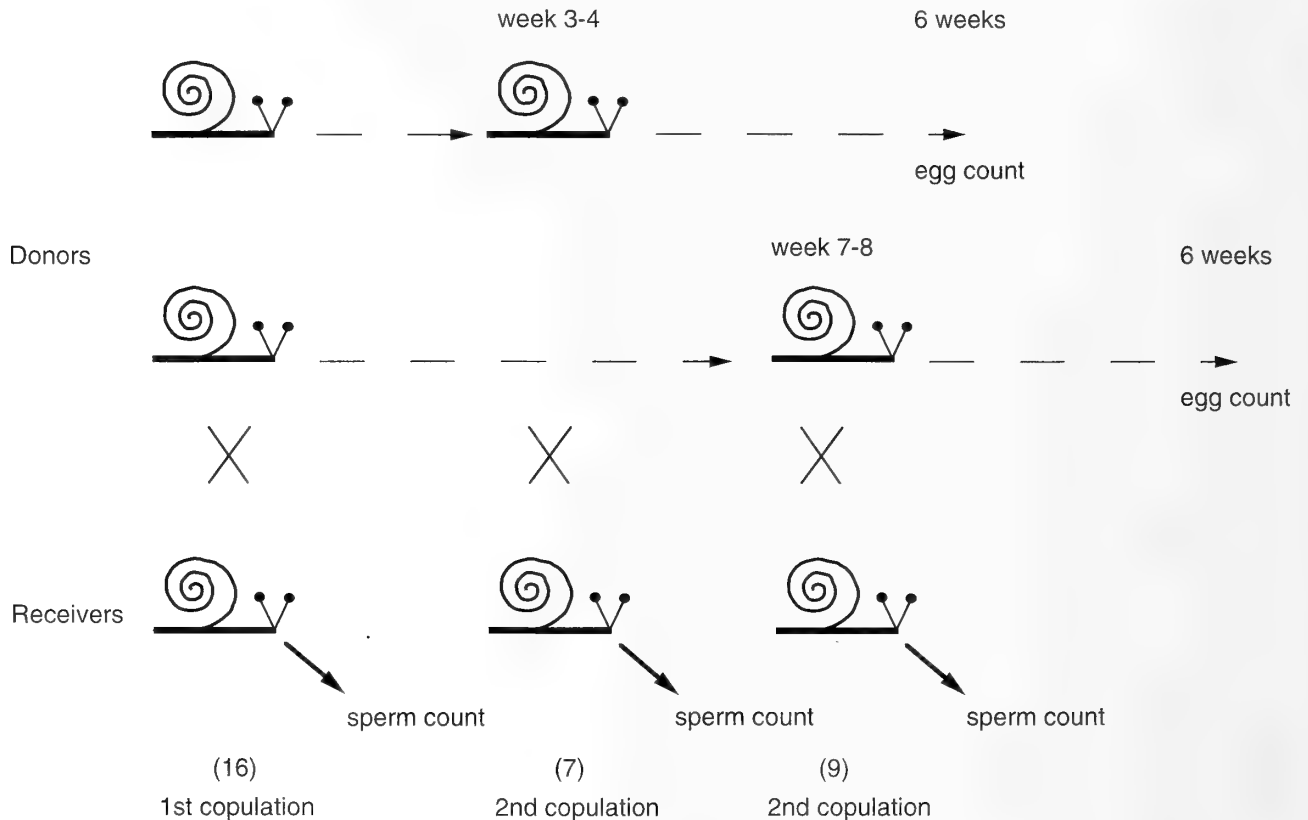


Figure 1. Design of mating experiment with sample size in parentheses.

shell volume =  $0.312 \times [(\text{breadth})^2 \times \text{height}] - 0.038$  (measurements in mm; B. Baur, unpublished data). Shell volume is a more reliable measurement of snail size than weight because weight depends on the state of hydration and thus is highly variable in terrestrial gastropods.

To obtain the spermatophore we dissected out the female reproductive duct of the recipient. The length (L) and width (W) of the sperm-containing part of each spermatophore were measured to the nearest 0.1 mm using a dissecting microscope. Spermatophore size (in  $\text{mm}^3$ ) was approximated, by the formula ( $\pi LW^2/4$ ), assuming a cylindrical volume. Spermatophores were kept singly in Eppendorf tubes at  $-30^\circ\text{C}$  until required.

The beakers of sperm donors were checked twice per week for eggs. The eggs of each batch were collected, counted, and kept in a plastic dish (6.5 mm in diameter) lined with moist paper towels at  $19^\circ \pm 1^\circ\text{C}$  to determine hatching success. Newly hatched snails were separated from remaining unhatched eggs to prevent egg cannibalism (Baur, 1992b). In both groups of snails, eggs were collected over a period of 40 days following the second copulation.

#### Sperm Counting Procedure

The number of sperm that an individual delivered was assessed by counting the number of sperm in the sper-

matophore transferred. This procedure is described in detail by Locher & Baur (1997). The spermatophore of *A. arbustorum* consists of a hardened secretion which encapsulates the spermatozoa (Hofmann, 1923). We mechanically disrupted the spermatophore in 200  $\mu\text{l}$  PBS-buffer (138.6 mM NaCl, 2.7 mM KCl, 8.1 mM  $\text{Na}_2\text{HPO}_4 \times 2\text{H}_2\text{O}$  and 1.5 mM  $\text{KH}_2\text{PO}_4$ ) using a pair of microscissors. The sperm suspension was homogenized with a set of Gilson pipettes for 5–15 min. To count the sperm, the homogenate was stained for 1–3 hr with an equal volume of a gallocyanin-chromium complex which stains the DNA in the head of the spermatozoa. If spermatozoa still occurred in clusters, we treated the sample overnight with a sonicator (35 kHz). Two subsamples of known volume of the sperm suspension were diluted 1:3 with PBS-buffer and transferred to a Bürker-Türk counting chamber. This counting chamber consists of 16 cells each with a volume of 25 nL. We counted all sperm heads in randomly chosen cells until the total number of sperm heads exceeded 400 and used the average of two subsamples to calculate the total number of sperm in a spermatophore.

#### Data Analyses

The StatView program package (Version 5.0, Abacus Concepts, 1998) was used for statistical analyses. Means

Table 1

Mating behavior, sperm delivery, and female fecundity in *A. arbustorum* that remated either after 3–4 weeks or after 7–8 weeks. Data from the second copulation are shown (egg number, hatching success of eggs, and number of hatchlings relate to the entire experimental period). Mean values  $\pm$  SE are presented. *P*-values result from unpaired *t*-tests.

Trait	Length of intermating interval		<i>t</i>	<i>P</i>
	3–4 weeks ( <i>n</i> = 7)	7–8 weeks ( <i>n</i> = 9)		
Time until initiation of courtship (min) <sup>1</sup>	107 $\pm$ 14	133 $\pm$ 33	0.17	0.86
Courtship duration (min) <sup>1</sup>	416 $\pm$ 45	263 $\pm$ 28	3.09	0.008
Copulation duration (min) <sup>1</sup>	120 $\pm$ 9	130 $\pm$ 13	0.49	0.63
Spermatophore volume (in % of spermatophore size in the first copulation)	87.0 $\pm$ 10.3	99.4 $\pm$ 6.3	1.07	0.30
Sperm (in % of sperm in the first copulation)	106.1 $\pm$ 18.1	110.2 $\pm$ 17.4	0.16	0.88
Total number of eggs produced <sup>1</sup>	64.4 $\pm$ 20.0	50.0 $\pm$ 10.1	1.23	0.24
Hatching success (%) <sup>2</sup>	64.7 $\pm$ 11.1	72.1 $\pm$ 7.4	0.70	0.50
Total number of hatchlings <sup>1</sup>	48.4 $\pm$ 15.4	38.3 $\pm$ 9.2	1.22	0.24

<sup>1</sup>  $\log_{10}$ -transformed.

<sup>2</sup> *arcsine*-transformed.

$\pm$  1 SE are given unless otherwise stated. We only considered snails that copulated twice and set the significance level  $\alpha$  at 0.01 to compensate for the large number of statistical tests based on data of the same individuals. To improve normality, some variables were  $\log_{10}$ - or *arcsine*-transformed.

## RESULTS

### First Copulation

The size of the spermatophore delivered during the first copulation varied from 1.93 to 3.84 mm<sup>3</sup> ( $\bar{x} \pm$  SE = 2.74  $\pm$  0.16 mm<sup>3</sup>, *n* = 16). The number of sperm transferred in the first copulation ranged from 1,281,800 to 3,599,700 (2,151,000  $\pm$  165,600, *n* = 16) and tended to be positively correlated with the size of the spermatophore ( $r$  = 0.53, *n* = 16,  $P$  = 0.032). Furthermore, spermatophore size tended to be positively correlated with the shell size of the sperm donor in the first copulation ( $r$  = 0.51, *n* = 16,  $P$  = 0.044). However, no correlation was found between number of sperm delivered and the shell size of the sperm donor ( $r$  = 0.04, *n* = 16,  $P$  = 0.89). Similarly, no correlation was found between spermatophore size, respectively, sperm number and the shell size of the sperm recipient in the first copulation (spermatophore size:  $r$  = 0.49, *n* = 16,  $P$  = 0.0513; sperm number:  $r$  = 0.16, *n* = 16,  $P$  = 0.56).

Virgin snails needed 105 min (median, range 30–360 min, *n* = 16) to initiate courtship. The median courtship time was 285 min (range 180–540 min, *n* = 16) and the median copulation duration was 150 min (range 90–300 min, *n* = 16). Neither courtship nor copulation duration was significantly correlated with the number of sperm transferred in a spermatophore (Spearman rank correla-

tion: courtship  $r_s$  = 0.27, *n* = 16,  $P$  = 0.29; copulation  $r_s$  = -0.14, *n* = 16,  $P$  = 0.59).

### Effect of Intermating Interval

Snails that remated after 3–4 weeks did not differ in shell size from those that remated after 7–8 weeks (mean shell volume of both groups: 1.24 cm<sup>3</sup>, range: 1.07–1.39 cm<sup>3</sup>;  $t$  = 0.09, *df* = 14,  $P$  = 0.93). There were differences in mating behavior between the two groups of snails. Courtship duration was shorter in individuals that remated after 7–8 weeks than in snails that remated after 3–4 weeks (Table 1). However, time until initiation of courtship and copulation duration did not differ between snails of both groups (Table 1). Furthermore, mating propensity (percentage of snails that mated in the trials) did not differ between the two groups (7.1% vs. 10.3%;  $\chi^2$  = 0.66, *df* = 1,  $P$  > 0.4). Compared with the first copulation, however, the average mating propensity was lower in the second copulation (8.7% vs. 22.0%;  $\chi^2$  = 13.29, *df* = 1,  $P$  < 0.001).

The difference in intermating interval did not affect spermatophore size and the number of sperm delivered in the second copulation (Table 1). Furthermore, individuals of neither treatment group differed in the number of sperm delivered in the first and second copulation (paired *t*-test; intermating interval 3–4 weeks,  $t$  = 0.17, *df* = 6,  $P$  = 0.87; intermating interval 7–8 weeks,  $t$  = 0.25, *df* = 8,  $P$  = 0.81). The number of sperm transferred in the second copulation averaged 2,130,000 (range 1,014,900–3,537,600, *n* = 16). No correlation was found between number of sperm delivered in the second copulation and the shell size of the donor and that of the receiver (donor:  $r$  = 0.20, *n* = 16,  $P$  = 0.46; receiver:  $r$  = 0.12, *n* = 16,  $P$  = 0.66). Furthermore, snails of both treatment groups



did not differ in female reproductive success (number of eggs produced and hatching success of eggs; Table 1). Moreover, female reproductive success did not differ between the two groups of snails when only a period of 40 days following the second copulation is considered (*t*-tests, in all traits  $P > 0.36$ , data not shown).

## DISCUSSION

The present study showed that the number of sperm transferred in a copulation of *A. arbustorum* did not increase when the intermating interval was prolonged from 3–4 to 7–8 weeks. This finding supplements the results of a previous study which indicated that individuals of *A. arbustorum* require at least 8 days to replenish their sperm reserves after a successful copulation, and that there is a slight increase in number of sperm delivered in the second copulation when the intermating interval extends to 22–29 days (Locher & Baur, 1999).

In simultaneously hermaphroditic opisthobranchs and pulmonates, the ovotestis produces both spermatozoa and ova, sometimes but not always simultaneously (Duncan, 1975). In *Helix pomatia*, autosperm are stored in the seminal vesicle of the hermaphrodite duct throughout the year (Lind, 1973). Phagocytosis of autosperm by the hermaphrodite duct epithelium has been reported in *H. pomatia* and *Oxychilus cellarius* (Müller, 1774) (Rigby, 1963). Sperm can be expelled from the hermaphrodite duct at times other than copulation to be eventually digested (as are foreign sperm) in the bursa copulatrix. In this way, unfertile and old spermatozoa can be recycled.

In many animal species, sperm number is an important determinant for achieving successful fertilization in sperm competition (Birkhead & Møller, 1998). In gastropods with internal fertilization, sperm are transferred to the partner in the form of free sperm, i.e., as sperm suspension in seminal plasma, or the sperm are either aggregated into loosely assembled naked conglomerates (spermatozeugmata) or encapsulated into spermatophores (Mann, 1984). However, little information is available about the number of sperm delivered in different gastropod species. In the sea hare *Aplysia parvula* Guilding in Mörch, 1863, the number of sperm transferred is positively correlated with copulation duration. When mating duration increased from 2 to 47 min, the number of sperm transferred increased from  $1 \times 10^5$  to  $6 \times 10^6$  (Yusa, 1994). In *Aplysia kurodai* Baba, 1937, and *A. juliana* Quoy & Gaimard, 1832, the number of fertilized eggs laid by an individual, which was allowed to mate only once, is positively correlated with copulation duration (Yusa, 1996). The ratio of transferred sperm to fertilized eggs is approximately 30:1 (Yusa, 1996).

Most freshwater pulmonates transfer a seminal fluid in which sperm is embedded (Geraerts & Joosse, 1984). During one copulation, the freshwater pulmonate *Bulinus globosus* ejaculates at least 350,000 sperm (Rudolph,

1983). *Bulinus globosus* (Morelet, 1866) is able to copulate as male once per day for up to 8 consecutive days. Following a single copulation, after 1 week of isolation, the hermaphroditic duct of male-acting individuals contained an average of  $87,000 \pm 42,000$  (SD) sperm. In the 10 days following the initial copulation, the snails produced approximately 50,000 sperm per day.

*Arianta arbustorum* transfers its spermatozoa in spermatophores. The ratio of transferred sperm to fertilized eggs is approximately 50,000:1, if one copulation is considered, or 25,000:1, if two copulations are considered. These figures significantly exceed the corresponding ratio recorded in *Aplysia* (see above). The present estimates of sperm number coincide with estimates of two independent studies using snails from the same subalpine population: the number of sperm delivered averaged 2,185,100 ( $n = 91$ , range 802,620–3,968,800) in Baur et al. (1998) and 2,573,000 ( $n = 31$ , range: 907,000–5,825,000) in Locher & Baur (1999). In contrast, lower numbers of sperm were transferred in three *A. arbustorum* populations in the Austrian Alps (mean values: 1,707,000 ( $n = 14$ ), 1,615,000 (15), and 1,802,000 (14), respectively; Baminger et al., 2000). However, the latter estimates were obtained from snails copulating in the wild, while higher sperm numbers were observed in animals kept under laboratory conditions. It is possible that geographical variation in number of sperm delivered exists in *A. arbustorum*. The production of sperm will certainly vary depending on the environment, the age, size, and nutritional state of the snail and most probably on the level of sperm competition.

In the present study, snails showed a higher mating propensity when they were allowed to copulate for the first time in June (22.0%) than when they were allowed to remate in July or August (8.7%). We used the percentage of individuals that mated in the trials as a measure of mating propensity. This is an indirect measure of mating frequency. A previous study showed that the more active a snail is, the more likely it will initiate courtship (Baur & Baur, 1992). Using similar experimental procedures, mating propensity ranged from 10.0% to 33.3% in different *A. arbustorum* populations (Baur & Baur, 1992). In natural populations, mating frequency decreases after the peak period (May to June). A similar seasonal decrease in mating propensity was observed in the present experiment. Most interestingly, snails prevented from remating for 7–8 weeks showed a shorter courtship duration than those prevented for 3–4 weeks. This difference cannot be explained by different conditions in the mating trials between the two experimental groups, as air temperature was similar during the test nights (Hänggi, 2000). On the other hand, seasonal effects on courtship duration cannot be ruled out. Courtship duration might also be short if there is no (or little) conflict between the gender roles in hermaphroditic mating partners (see Michiels, 1998). In *A. arbustorum*, copulations toward the

end of the reproductive season may mainly serve to replenish allosperm reserves in the storage organ as no further eggs are produced. In this situation, little sexual conflict may occur between the gender roles. This hypothesis needs testing. However, individuals prevented from remating for a longer period did not differ in female fecundity (number of eggs produced and hatching success of eggs) from those that remated earlier, confirming that one successful copulation per reproductive season is sufficient to fertilize all the eggs produced by an individual (Chen & Baur, 1993).

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## On the Adaptive Function of the Love Dart of *Helix aspersa*

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**Abstract.** The phenomenon of “dart shooting” in land snails of the genus *Helix* remains unexplained from an adaptive viewpoint. Data on the sexual behavior of *Helix aspersa* and *H. pomatia* compiled from previous accounts, supplemented with new observations, do not support the traditional hypotheses that the dart serves reproductive isolation or behavioral stimulation/coordination functions. For example, successful copulation does not depend on dart receipt. An alternative class of explanations for dart shooting is considered: sexual selection. Sexual selection hypotheses for dart function, including certainty of parenthood, male manipulation, and female choice (by both fisherian runaway and indicator mechanisms) are reviewed and compared against the observational evidence. The theory of female choice by handicap/indicator/good genes processes is implemented to propose that dart shooting is a male sexual signal used by females to select sperm from among sperm donors. Male manipulation and female choice are not easily distinguishable as adaptive explanations of dart shooting.

### INTRODUCTION

The sexual behaviors exhibited by many hermaphroditic land snails (Gastropoda, Pulmonata, Stylommatophora) are well known for their complexity and vigor. A bizarre feature is the sometimes forceful deployment of calcareous spines and darts, as well as of non-calcareous organs. The gross anatomy of these organs and, in some cases, the behaviors associated with them, have been described for many species (references below). It is therefore surprising that these features have not been better investigated from a behavioral ecological perspective. In fact, empirically supported adaptive explanations for their function are lacking.

This is not to say that zoologists have been uninterested in snail sexual behavior and reproduction. The occurrence and natural history of spine, “dart,” and “stimulator” (sarcobelum) use in land snails were noted even before Ashford’s (1883) monograph describing darts and associated structures in British helicids (references in Kothbauer, 1988). Subsequent descriptions for stylommatophoran pulmonates include: for *Helix*, *Cepaea*, *Theba*, *Tacheocampylaea*, *Eobania*, and *Arianta* (family Helicidae): Meisenheimer (1907, 1912), Szymanski (1913), Taylor (1914), Hofmann (1923), Dorello (1924), Graefe (1962), Herzberg & Herzberg (1962), Petersen (1971), Lund (1971), Lind (1976), Jeppesen (1976), Giusti & Lepri (1980), Chung (1987), Beaumont (1988), Giusti & Andreini (1988), and Adamo & Chase (1988); for *Phenacolicolimax* (Vitrinidae), *Milax* (Milacidae), *Arion* (Arionidae), *Parmacella* (Parmacellidae), and *Limax* and *Dero-*

*ceras* (Limacidae): Adams (1898), Gerhardt (1933, 1935, 1940), Quick (1946), Webb (1950), Langlois (1965), Rymzhanov & Schileyko (1991), and Reise (1995); for *Ventridens* (Zonitidae): Webb (1948); for *Helminthoglypta* (Helminthoglyptidae): Webb (1951, 1952); for *Partula* (Partulidae): Lipton & Murray (1979); for *Gymnarion* (Urocyclidae): Binder (1976); for *Euglandina* (Streptaxidae): Cook (1985); and for *Philomycus* (Philomycidae): Webb (1968) and Tompa (1980). Most of these studies were carried out from a perspective of proximate mechanism rather than ultimate adaptation, if function was considered at all. Nonetheless, several hypotheses have been put forward to provide adaptive explanations for snail precopulatory behaviors, and specifically for dart shooting (Dorello, 1925; Diver, 1940; Goddard, 1962; Lind, 1976; Charnov, 1979; Tompa, 1980, 1984; Chung, 1987; Giusti & Andreini, 1988; Leonard, 1991, 1992; Adamo & Chase, 1990, 1996; Baur, 1998). None has received unambiguous empirical support.

This paper has three main purposes: (1) I first describe the sexual behavior of *Helix aspersa* (Müller, 1774) and *H. pomatia* (Linnaeus, 1758). (2) I then review and comment on some of the published hypotheses purporting to explain dart function in these species. Most of these are not wholly consistent with the observed behavior and biology, whereas others may be plausible but remain untested. (3) Lastly, I apply the hypothesis of female choice based on male sexual signals (Andersson, 1994; Charnov, 1979; Fisher, 1915) as an adaptive explanation for dart shooting in *Helix*: dart shooting is a male sexual signal used by females as the basis upon which to select sperm from among several mates. Females choose the fathers of their offspring based on their perception of their mates’ dart shooting effectiveness. However, whereas Charnov

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(1979) explained female choice based on the dart using the fisherian runaway mechanism, I invoke the concept of indicators for "good genes" ("handicaps," Andersson, 1994; Grafen, 1990a, b; Iwasa et al., 1991; Zahavi, 1975, 1977; Zahavi & Zahavi, 1997). The hypothesis is consistent with the known behavior and reproductive biology, but is as yet untested. Although stylommatophorans are simultaneous reciprocal hermaphrodites, I distinguish "male" and "female" attributes throughout this paper.

## METHODS

*Helix aspersa* individuals were collected in Dublin, Ireland and Berkeley, California, USA and purchased from Blades Biological (collected in County Kent, United Kingdom) in March and April 1996. Additional specimens were collected in Vienna, Austria in September of the same year. Within 1 week of receipt, all snails received a unique number painted onto the shell with white non-toxic paint, and were isolated in individual clear plastic half-liter cups with tight-fitting lids. Cups contained 2–3 cm of sand and potting soil mixture. Throughout the experimental period (May 1996–September 1997) the animal cages were cleaned and the snails showered and fed twice weekly with raw vegetables (lettuce, cucumber, carrot, cabbage); crushed oyster shells were provided *ad libitum*.

A total of 246 sexually mature snails, identified as such by the presence of a reflected shell lip, were subjected to a surgical procedure to check for possession of a dart in the dart sac and for the presence of macroendoparasites of the accessory sexual organs. For surgeries, snails were anesthetized/immobilized with 0.1 ml/g of body weight of 0.01% succinyl choline chloride and 4% MgCl<sub>2</sub> with 0.005% added streptomycin (Chung, 1985). An approx. 0.75 cm incision was made in the right side of the body wall approx. 1 cm behind the genital aperture. The dart sac and digitiform glands were lifted out through the incision with a polished glass probe and examined under low magnification with a dissecting microscope. In 106 out of the 246 snails (43%), the dart sac and digitiform glands (Figures 5, 7, 8) were excised; the remainder of the surgeries were sham manipulations. In all surgeries, two sutures were inserted to facilitate wound healing. Snails usually regained body turgor and mobility within 18 hours. Survivability 1 week following the surgery was 83%; there was no significant difference in survivability between dart sac and digitiform-excised and sham-operated snails.

Sexual encounters of *H. aspersa* were staged by introducing 6–12 showered snails into a 10 L plastic basin. Pairs displaying signs of sexual receptivity were removed to a smaller clear plastic cup where they were continuously and closely observed until they either broke off sexual behavior or achieved successful copulation. The occurrence and timing of all behaviors, including dart

shooting and receipt, were recorded for 156 sexual encounters.

Nineteen *H. pomatia* sexual encounters were observed and recorded in the field on the grounds of the Max Planck Institute for Behavioral Physiology near Starnberg, Germany during the spring and summer of 1997. No dissections were performed on *H. pomatia*.

## DART-SHOOTING BEHAVIOR IN *HELIX*

The sexual behavior and reproductive biology of *Helix aspersa* and *H. pomatia* (Helicidae, Helicinae) have been well documented. The account given here of *H. aspersa* and *H. pomatia* matings is similar to those of Meisenheimer (1907), Jeppesen (1976), and Giusti & Lepri (1980); Lind (1976), Chung (1987), and Adamo & Chase (1988) gave more quantitative descriptions. The first and second parts of this section cover the precopulatory and copulatory behaviors of *H. aspersa* and *H. pomatia*, whereas the third part addresses additional matters concerning dart shooting and the biology of *Helix*. Results not attributed to previous works have been derived from my own dissections and observations of sexual encounters.

### Sexual Behavior

Upon meeting, two receptive *H. aspersa* raise their heads slightly and engage in bouts of mutual facial caressing, mouth-mouth and mouth-genital pore contact, and biting. These behaviors may be punctuated by interruptions (1–20 min in duration) in which the snails separate and/or circle clockwise about one another. The extensive physical contact during this initial phase ("introductory behavior," Lind, 1976) may allow each snail to gather information via mechanical and/or chemical cues regarding the species, size, health, and/or sexual receptivity of its prospective partner. "Receptivity" is here only loosely defined. A receptive snail attempts to initiate sex with every snail that it contacts; other receptive individuals respond to advances from any initiator, whereas non-receptive snails shun such advances. Although subtle precopulatory mate choice may exist in *Helix* and other helicids, there is no direct evidence for it (but see Fearnley, 1996). For example, Baur (1992) and Baur & Baur (1997) found that matings in *Arianta arbustorum* (Helicidae, Ariantinae) occur randomly with respect to shell size and degree of relatedness, respectively.

Within 1–12 minutes after contact between receptive individuals, the genital pore region (GPR), on the right side of the head, becomes swollen. The genital atrium partly everts, and each snail presses its GPR against that of its partner (Figure 1). The mutual GPR contact alternates with mouth-mouth and mouth-GPR contact, biting, and pauses. *Helix aspersa* pairs maintain a side-to-side posture, and although they may raise their heads slightly, each snail retains substrate contact with nearly the entire



Figures 1–4. Courtship of *Helix aspersa*. Images are from different courtship sequences. For scale, snail shells are approx. 2.7 cm along their longest dimension. Figure 1. Early in courtship; neither snail has shot its dart. The genital pore regions (GPRs) of both snails are swollen and everted (white tissue masses between snails' right tentacles), and each snail presses its GPR against that of its partner. Figure 2. The left snail has just ejected its dart (white pointed object emerging from right side of head). The dart did not strike the partner. Figure 3. Unilateral copulation attempt; the snail on the right has everted its penis. The swollen GPR of its partner (to immediate left of penis) is apparent. Figure 4. Successful mutual copulation. Intromission was achieved approx. 2 min before photo was made. The shot dart of the lower snail can be seen protruding from the recipient.

foot (Adamo & Chase, 1988; Chung, 1987). In contrast, *H. pomatia* partners press the front one-third to one-half of the ventral surfaces of their feet together in a "sole-to-sole" posture, raising their heads in a "frontal upright" position (Meisenheimer, 1907; Lind, 1976).

After a variable length of time (in *H. aspersa*, 5–90 min following initial GPR swelling; personal observation), one partner performs dart shooting by forcibly ejecting its dart from the genital pore (Figure 2). As *Helix aspersa* partners are usually in GPR-GPR contact at the time of dart shooting, the shot dart often hits the recipient in or near the GPR; a well aimed and forcefully expelled dart may penetrate the partner's body wall. If the shooter's GPR is not appressed to the partner upon shooting, the dart may miss the intended recipient or strike it without penetrating.

Most sexual behaviors in *H. pomatia* proceed more slowly than in *H. aspersa*, and the postures differ (Jepesen, 1976; Lind, 1976). Prior to dart shooting, and starting from the sole-to-sole, frontal upright posture, *H. pomatia* lowers its head, usually pressing its GPR against the sole of its partner. Lind (1976) noted that *H. pomatia* appears to perform orientation movements before dart shooting. He observed that these movements do not "lead the atrium (GPR) to a specific goal for the dart," but rather ensure that the shooter's GPR is appressed against the partner such that the dart strikes the latter upon being shot. The location of striking and/or penetrating darts differs between the two species, reflecting the different postures of the partners at the time of dart shooting: in *H. aspersa* most darts (60%, Chung, 1987) hit the recipient in or near its GPR, whereas *H. pomatia* darts usually hit

the sole of the foot (75%, Lind, 1976). However, as the partners are not always aligned exactly as described above during dart shooting, darts are received in both species in the head, mantle, penis, body side wall, and foot. Some shot darts miss the intended recipient entirely (8–10%, Adamo & Chase, 1988; Koene & Chase, 1998a; 12%, personal observation; all data from *H. aspersa*). The response to being shot ranges from no observable reaction to a brief (< 30 sec in *H. aspersa*) recoil away from the stimulus, although in rare cases (< 2% of pairings, Chung, 1987) the encounter may be terminated.

In *H. aspersa*, immediately after the first partner shoots its dart, that snail begins attempting intromission (Figure 3): it presses its swollen GPR against that of the partner and periodically (approx. 1/1–5 min) everts its penis. The snail which has not yet shot its dart appears not to allow unilateral intromission; nor does it commence penis eversions itself. Instead, a variable period elapses during which the first dart-shooter attempts intromission unilaterally while the second snail remains in the “pre-shooting” stage, in which it presses its GPR against its partner without everting its penis (Chung, 1987; Adamo & Chase, 1988; personal observation). Zero to 120 minutes after the first dart-shooting event, the second snail shoots its dart, after which it too commences penis eversions. The sequence and variability of dart shooting and the initiation of intromission attempts are qualitatively similar in *H. pomatia* (Lind 1976; personal observation).

### Copulation and Spermatophore Transfer

In *H. aspersa* pairs, near-simultaneity of bilateral intromissions appears to be required for successful copulation, as each snail appears not to allow intromission by its partner unless it also achieves intromission. (This is discussed below in the section “Existing Hypotheses for *Helix* Dart Function, Sexual Selection/Conflict Hypotheses.”) In order to achieve successful mutual intromission, it appears necessary that the interval within which *H. aspersa* partners execute simultaneous penis eversions is 5–6 sec or less (personal observation). In addition to near-simultaneity, successful bilateral intromission appears also to require proper orientation of the partners, such that both penes are properly “aimed.” As a result of the apparent difficulty in accomplishing this feat, the time between the onset of mutual intromission attempts and successful mutual copulation is quite variable, in the range 1–120 min for *H. aspersa*. Partners usually perform multiple penis eversions before achieving mutual copulation; Adamo & Chase (1988) reported a mean of 11 per copulant in *H. aspersa*. Nonetheless, undisturbed pairs that have begun mutual copulation attempts usually succeed (95% of cases in *H. aspersa*,  $n = 65$  pairs, personal observation). Once successful mutual intromission occurs, *H. aspersa* copulants become quiescent and assume a stereotypical posture with the tentacles extended but

flaccid (Figure 4). After approx. 15–30 min, the tentacles are fully withdrawn and the snails maintain almost complete quiescence throughout the duration of copulation (Adamo & Chase, 1988; personal observation). Copulation in *H. pomatia* differs, and is described below.

*Helix aspersa*, *H. pomatia*, and many other helicids do not transfer naked sperm during copulation, but rather package it within a spermatophore (*H. pomatia*, Lind, 1973). In *H. aspersa*, formation of the single spermatophore (per snail) begins in the epiphallus and flagellum within 2 minutes after intromission (Adamo & Chase, 1988). Filling of the spermatophore follows, and the sperm-filled spermatophore enters the penis 2–5 hr after the onset of copulation. Bidirectional spermatophore transfer is completed after approx. 5–6 hr, and copulation lasts 6–8 hr (*H. aspersa*, Adamo & Chase, 1988). Occasionally (8% of pairings, personal observation) partners separate before one or both spermatophores have been completely transferred; 0–5 cm of allospermatophore tail can then be seen dangling from the genital pore. The tail is taken up over the next 1–2 hr, but variable lengths of its tip may break off before uptake (10% of 105 spermatophore transfers, personal observation for *H. aspersa*).

The apparent mutual enforcement of mutual intromission and its resulting near-simultaneity apply also to *H. pomatia*, but other aspects of copulation and spermatophore transfer differ in this species: copulation lasts just 5–10 min, and only the spermatophore head and body are transferred during intromission. Formation and filling of the spermatophore are accomplished in < 5 min (Lind, 1976). Afterward, the penes are withdrawn and the partners remain immobile and in sole-to-sole contact while the allospermatophores are actively taken up by both snails. Spermatophore tails can easily be seen being transferred for several hours following withdrawal of the penes. Each partner (*H. pomatia*) aids spermatophore transfer by generating distally directed muscular waves of contraction of its sole (Lind, 1976; personal observation). Complete uptake requires up to 9 hr (mean 5.5 hr, Lind, 1976), during which the snails remain quiescent.

The spermatophore consists of a head/neck, a body holding the sperm, and a long tail (Meisenheimer, 1907; Lind, 1973; Adamo & Chase, 1988). The spermatophore of *H. aspersa* is 12–15 cm long (Adamo & Chase, 1988), whereas that of *H. pomatia* is 10–12 cm (Lind, 1973). In *H. aspersa* the spermatophore is placed into the receiving snail's bursa diverticulum (Figure 5). *Helix pomatia* lacks a bursa diverticulum, and in this species the spermatophore is accepted instead into the bursa copulatrix. During spermatophore transfer, the spermatozoa remain quiescent, but they become activated and begin to exit the spermatophore through its grooved tail approx. 45 min after copulation (*H. pomatia*, Lind, 1973). Individual sperm are approx. 850  $\mu\text{m}$  long (*H. pomatia*, Thompson, 1973).

Baur et al. (1998) found that the amount of sperm transferred in spermatophores of *A. arbustorum* was uncorre-



lated with the sizes of either donor or the partner, the duration of copulation, the partner's previous mating history (virgin or non-virgin), or the amount of sperm received. Control of sperm transfer amount would be expected if males exercise mate choice or if partners "trade" sperm, i.e., if sperm donation is conditional on sperm receipt.

During and after successful spermatophore transfer, the female reproductive tract generates peristalses that pull the allospermatophore into the bursa diverticulum (*H. aspersa*, Koene & Chase, 1998b) or bursa copulatrix (*H. pomatia*, Lind, 1973). In *H. pomatia* the spermatophore exceeds the receiving bursa copulatrix in length, but despite this length advantage it is caused to "crumple up" by the receiver's peristalsis and is pulled entirely into the tract approx. 6–18 hr after copulation (Lind, 1973). The bursa complex functions not to store transferred allosperm but, by the secretion of digestive enzymes, to break down the spermatophore and destroy the sperm remaining in it. In order to escape digestion and to be stored for possible later fertilization of eggs, spermatozoa must move against the peristalsis to reach the spermatheca (sperm storage organ) at the opposite end of the spermoviduct. Distally directed peristalsis of the spermoviduct further retards the migration of allosperm (Figure 5; Lind, 1973). As the reproductive tract may actively inhibit allosperm from reaching the spermatheca, variation in the strength and/or frequency of peristaltic activity could influence the amounts of transferred sperm which are stored vs. destroyed. Curiously, Chen & Baur (1993) and Locher & Baur (2000) found that 8% and 10%, respectively, of *A. arbustorum* failed to lay fertile eggs following a single successful copulation, and in my own experiments with *H. aspersa*, 21% of twice-mated snails ( $n = 29$ ) failed to lay eggs over a subsequent 2-month period. There are many reasons why snails might not lay fertile eggs after copulating, but one possible reason is that none of the sperm received during those copulations was successfully stored.

The anatomy of the spermatheca indicates that the separate storage and/or retrieval of sperm from different matings is at least a possibility. The spermatheca is composed of three to six blind-ended tubules, the walls of which are muscular and lined with ciliated epithelium (*H. pomatia*, Lind, 1973; *H. aspersa*, Brisson et al., 1977). The spermathecae of the helicine helicids *Eobania vermiculata*, *Tacheocampylaea tacheoides*, *H. aperta*, *H. lucorum*, and *Theba pisana* also consist of "one or more blind sacs" (Giusti & Andreini, 1988). The spermatheca is best studied in *A. arbustorum*, in which allosperm are stored in a spermatheca of similar gross structure (two to nine tubules, Haase & Baur, 1995; Baminger & Haase, 1999; Baminger et al., 2000). It is not yet known if sperm from different matings are stored in separate tubules, but it is clear that individuals can use several tubules for sperm storage (Baminger & Haase, 1999). Further, the intricate musculature, innervation, and ciliation of the spermatheca suggest the capacity to control allosperm movements into

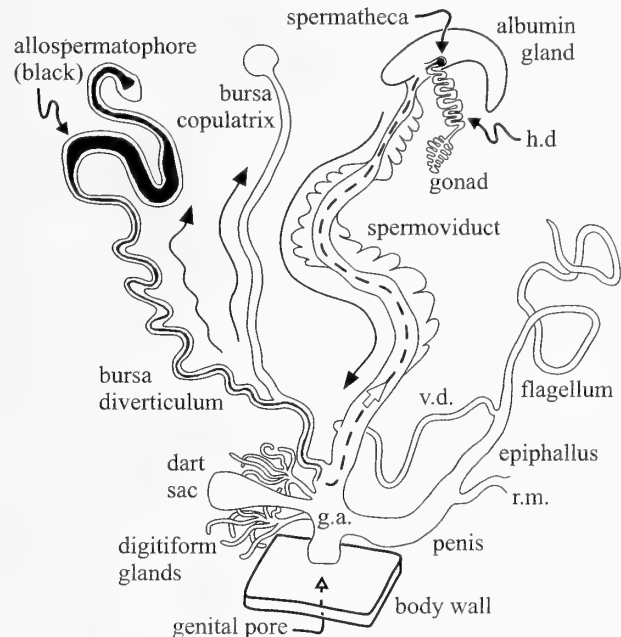


Figure 5. The reproductive organs of *Helix aspersa*, depicted immediately after successful spermatophore receipt. The allospermatophore (black) has been received from the partner into the bursa diverticulum. The thickened portion of the spermatophore is the body, holding the sperm; its tail extends down into the genital atrium. The dotted line (hollow arrowhead) running internally from the genital atrium up the spermoviduct to the spermatheca is the path along which allosperm must traverse in order to be successfully stored. Solid lines with filled arrowheads, drawn outside of the organs, depict reproductive tract peristalses directed against the pathway taken by successfully-stored sperm. A snail's own spermatophore (not shown) is formed and filled in the epiphallus and flagellum and transferred via the everted penis (here shown retracted). g.a., genital atrium; h.d., hermaphroditic duct; r.m., penis retractor muscle; v.d., vas deferens.

and/or out of individual tubules (Bojat et al., 2001). Stored sperm remain viable for at least 1 year in *A. arbustorum* (Baur, 1988) and *Cepaea nemoralis* (Helicidae, Helicinae, Murray, 1964).

Fertilization occurs at oviposition, which can take place from 1 day to many months after copulation (*H. pomatia*, Perrot, 1938 cited by Tompa, 1984). Fertilization occurs in a pouch located at the confluence of the hermaphroditic duct, the spermathecal duct, and the spermoviduct (Figure 5; better illustrated by Lind, 1973). Allosperm are released or transported from the spermatheca to the fertilization pouch, but it is unknown if snails can control the amounts of sperm released from the different spermathecal tubules (see previous paragraph and Bojat et al., 2001). *Helix aspersa* may produce from zero to several clutches following a single mating (Moulin, 1980). The number of matings achieved by wild individuals of *H. aspersa* is unknown, but is likely to be on the order of two to six per year (Baur, 1998). Tischler (1973)

reported that snails of a small population of *H. pomatia* in northern Germany copulated at least two to four times per season, and Lind (1988) found that individuals in his larger Danish population of the same species mated at least five to six times annually. Murray (1964) and Baur (1994) reported that the offspring of wild individuals of *Cepaea nemoralis* and *A. arbustorum*, respectively, were fathered by more than one individual.

The behaviors of some other helioid species may be interpreted similarly. Giusti & Lepri's (1980) and Giusti & Andreini's (1988) behavioral descriptions of *Eobania vermiculata*, *Tacheocampylaea tacheoides*, *H. aperta*, *H. aspersa*, *H. lucorum*, and *Theba pisana* are less quantitative than those of Lind (1976), Chung (1987), and Adamo & Chase (1988), but are qualitatively consistent with the accounts given in those reports and here. The one notable exception is that dart shootings were observed to occur simultaneously in pairs of *E. vermiculata*, *T. tacheoides*, and *T. pisana*; the significance of the differences in simultaneity of dart shootings among species is unknown. In all species studied by Giusti and colleagues, copulation success appeared not to depend on dart receipt.

### Dart Characteristics

The following aspects of dart shooting are discussed because of their potential relevance to the dart's adaptive function: (i) the fate of a shot dart, (ii) dart composition and regeneration, (iii) substances transferred by the dart, (iv) common snail parasites, and (v) variability in dart shooting and receipt.

(i) Shot darts do not always strike the partner, and even when they do, they do not always penetrate the body wall. In *H. aspersa*, Chung (1987), Adamo & Chase (1988), and Koene & Chase (1998a) found that a shot dart penetrated the recipient's skin in 67–92% of cases. In the remaining instances the dart either struck the partner but did not penetrate or completely missed. For penetrating darts, the extent of penetration varies from superficial (< 1 mm) to deep (the entire 9 mm length); occasionally a dart is thrust so hard that its tip emerges from the other side of the recipient's body (Figure 4). The duration of penetration varies correspondingly. Deeply penetrating darts often remain lodged in the recipient's skin/body for many minutes to hours, and may even be absorbed (allodart absorption by the recipient occurred in 6–46% of penetrations in *H. aspersa*, Chung, 1987; Adamo & Chase, 1988; Koene & Chase, 1998a). Shallowly penetrating darts usually become dislodged and fall out within seconds or minutes (own data, unpublished). A dart that misses or falls out may simply lie on one of the snails or fall to the substrate.

In *H. aspersa*, of those darts not striking the intended target snail, many are shot so weakly that they are not fully expelled from the shooter's dart sac (Chung, 1987; personal observation). In these and other cases in which



Figure 6. A shot dart (length 9 mm) that missed the intended recipient. The dart base, with adhering digitiform gland mucus, is at bottom.

it fails to lodge in the recipient's skin, the dart may be retracted by the shooter. "Used" and retracted autodarts are held briefly in the dart sac or genital pore, but are later either expelled onto the ground or transferred to the bursa copulatrix/diverticulum (Chung, 1987; J. Koene, personal communication). In any case, a shot dart is not re-used, possibly because upon shooting it becomes physically decoupled from its connection to the "tubercle" within the dart sac. The tubercle, at the base of the dart sac, is the structure upon which the dart forms (Dillaman, 1981). This decoupling is thought to render a dart subsequently unusable (*H. aspersa*, D. Chung, personal communication; *H. pomatia*, Lind, 1973).

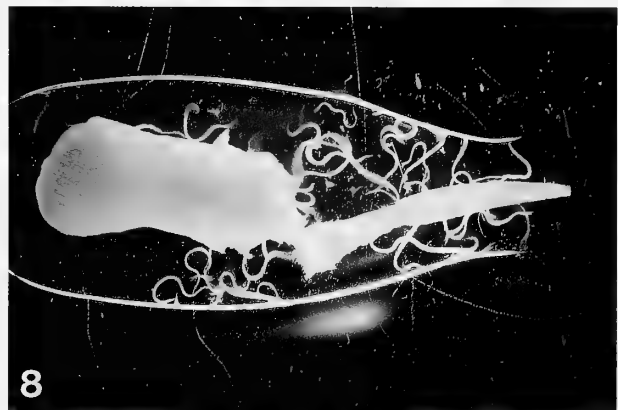
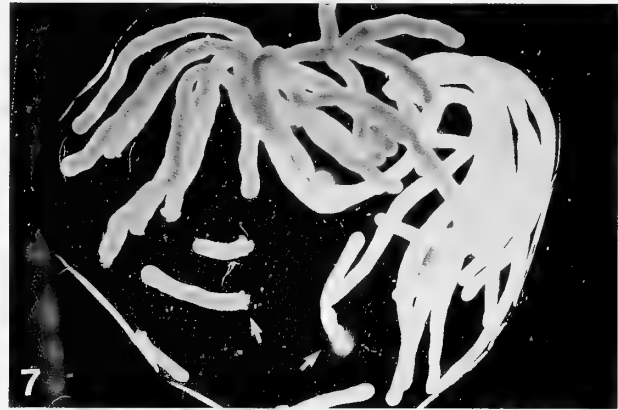
(ii) *Helix aspersa* and *H. pomatia* possess only one dart at a time. The dart of *H. aspersa* is approx. 9 mm long (Figures 6, 8) and made of aragonite ( $\text{CaCO}_3$ ) and a protein scaffold (Hunt, 1979; Dillaman, 1981). It bears four blades over most of its length; a cone-shaped base fits over the dart sac's tubercle. After dart shooting, 5–7 days are required for dart regeneration (*H. pomatia*, Jepsen, 1976; *H. aspersa*, Dillaman, 1981; Tompa, 1982). The amount of calcium in a dart (*H. aspersa*:  $0.37 \pm 0.13$  mg, Koene & Chase, 1998a) is approximately equal to that in a single egg, and the mean clutch size for this species is 50–60 eggs (range 20–130, Herzberg & Herzberg, 1962; Koene & Chase, 1998a). The relative amounts of calcium in darts and eggs and the fact that the dart is usually not absorbed by the recipient suggest that the dart calcium does not function as a nuptial gift (Koene & Chase, 1998a), a possibility raised by Charnov (1979) and Leonard (1991). Indeed, J. Koene (unpub-

lished) found that most of the calcium transferred in an absorbed dart is excreted.

(iii) Upon being shot, the dart is liberally coated by a white mucus (Figure 6) secreted by the paired digitiform glands; at least some of the mucus of a well penetrating dart enters the recipient's haemocoel (*H. aspersa*, Adamo & Chase, 1990). The composition of the mucus is unknown. Koene & Chase (1998b) found that *in vitro* application of the mucus induces contractions of the female reproductive tract. These contractions may influence the dart recipient's disposition of the shooter's sperm, an interpretation supported by results reported by Rogers & Chase (2001).

The size and apparent health (based simply on visual inspection) of digitiform glands vary among snails (Ashford, 1883; Taylor, 1914; personal observation). In field-collected adult *H. aspersa* from California, England, and Austria (the last introduced from ex-Yugoslavia, Reichütz, 1978; W. Fischer, personal communication), discolored digitiform glands were found in 2% ( $n = 92$ ), 23% ( $n = 92$ ), and 6% ( $n = 62$ ) of snails, respectively ( $n = 246$ ; this study). Normal glands are white, and discoloration ranges from yellow to brown (Figure 7). Discolored glands contain darker and more viscous mucus than do white, apparently healthy, glands. The discolored-gland condition was found only in non-virgins (personal observation), as determined by the snail's possession of a normal or virgin dart (Chung, 1986b), suggesting that the condition is transmitted venereally. Excluding virgins, the percentages of snails with discolored digitiform glands from the Californian, English, and Austrian populations were 6%, 29%, and 8% respectively (this study). In addition to the digitiform gland discoloration, the glands and/or dart sac bore dark cysts in 2% of the English and 6% of the Austrian snails (this study).

(iv) Wild *H. aspersa* from its native habitat hosts a variety of parasites, some in the reproductive tract. For example, the nematode *Nemhelix bakeri* inhabits the dart sac (Figure 8) and other reproductive organs; venereal transmission occurs via the transferred spermatophore (Morand, 1988) and possibly via dart shooting. Heavy infestations of *N. bakeri* cause decreased fecundity (*H. aspersa*, Morand, 1989). In my *H. aspersa* samples, nematodes were found in 32% of (native) English non-virgins ( $n = 22$ ), but in none of the (introduced) California and Austrian snails. Other parasites of land snail reproductive, digestive, or respiratory systems include the sporozoan *Klossia helicina* (Taylor, 1914), the ciliate *Myxophyllum steenstrupi* (Taylor, 1914), the flagellate *Cryptobia helices* (Lind, 1973), the trematode *Dicrocoelium dendriticum* (Taylor, 1914), the nematode *Rhabditis maupasi* (Brockelman & Jackson, 1974), and the mite *Riccardoella limacum* (Baker, 1970). These parasites affect host fitness (*H. aspersa*, Morand, 1989; Graham et al., 1995) and are often transmitted between snails during sexual encounters (personal observation for the mites on *H. aspersa*; Lind,



Figures 7 and 8. Diseases of the dart sac and digitiform glands. Figure 7. Digitiform glands, one healthy (white) and one diseased (gray; brown in life) from the same snail. Individual tubules were transected to show the consistency of the mucus. The gray mucus is much more viscous than the white mucus, demonstrated by the different extents of diffusion into the saline droplet (white arrows). Tubule widths are approx. 0.75 mm. Figure 8. Nematode parasites of the dart sac. The dart sac (left) was excised from a live snail and the dart (right) was expelled manually. Approx. 30 nematodes (*Nemhelix bakeri*) can be seen in the saline drop; the nematode images appear blurred because the worms were swimming. The white flask-shaped line is the edge of the saline droplet under transillumination. The dart is approx. 8 mm in length.

1973 found that the presence of *Cryptobia* in *H. pomatia* was correlated with previous sexual activity).

Variability in the health of the digitiform glands resulting from disease, parasites, and/or other factors, e.g., senescence, should contribute to the total variation in the amount and/or potential bioactivity of the digitiform gland mucus transferred by dart shooting.

(v) If a sexually active *H. aspersa* possesses a dart, it generally shoots it during precopulatory behavior. That is, for those individuals possessing a fully formed dart, dart shooting is *not* a conditional, i.e., optional behavior (Chung, 1987 and personal communication; personal observation); snails do not "decide" whether or not to shoot

a dart during a particular encounter. (But see Adamo & Chase [1988], who reported that *H. aspersa* failed to shoot a dart, even though individuals had one, in 5% of cases. For at least some reported cases of failures to shoot, it is possible that the dart had in fact been weakly shot and then retracted into the dart sac or genital atrium.) There are, however, two known circumstances in which *H. aspersa* individuals undergo an otherwise normal mating sequence *without* shooting a dart. In both cases, the reason for a snail's failure to shoot is that it lacks a dart. The first instance is that in which a snail mates within approx. 7 days after having shot its dart in a previous encounter. Snails mating in this interval have not yet regenerated a dart, and so do not shoot one. *H. aspersa* individuals are less likely to remate within a 2-day refractory period following copulation (Chung, 1987); *H. pomatia* will remate with either the same or a different individual, without dart shooting, within 3 days of copulating, after which it also experiences an approx. 5-day refractory period (Jeppesen, 1976; Lind, 1976). The second case of non-shooting occurs in virgin snails: in order to produce its first dart, a snail must apparently undergo precopulatory behavior, including the dart-shooting phase (Chung, 1986b). Virgin snails occasionally exhibit conspicuous dart-shooting behavior without expelling a dart; the dart sac is everted, but no dart is present (Adamo & Chase, 1988; personal observation).

In *A. arbustorum* the question whether individuals "decide" to shoot a dart in a given encounter is unresolved. Baminger et al. (2000) dissected snails post-copulation, checking for the presence and locations of shot and un-shot darts. It was found that 60.5% of mating snails held a fully formed dart in the dart sac after copulation, implying that dart shooting occurs in fewer than half of matings, per individual. However, Baminger et al. did not check whether darts found in the dart sac after copulation were detached from the tubercle (H. Baminger, personal communication). As discussed above, dart detachment from the tubercle appears in *H. aspersa* and *H. pomatia* to signify an "attempt" to shoot the dart. Except for Baminger et al. (2000), dart shooting has not been studied as well in *A. arbustorum* as it has in *H. aspersa* and *H. pomatia*. The available evidence and observations (e.g., *A. arbustorum*'s relatively larger digitiform glands secrete copious amounts of mucus, which is actively ingested by both partners prior to copulation; B. Baur, personal communication) suggest that dart shooting and mucus production function differently in *A. arbustorum* as opposed to *H. aspersa* and *H. pomatia*.

The above discussions highlight the fact that both dart shooting (occurrence, "force," and "aim") and receipt (occurrence, location, extent, and duration of penetration) are therefore quite variable (Lind, 1976; Chung, 1987; Koene & Chase, 1998a). Moreover, the differences in dart shooting effectiveness and in the quantity and quality of digitiform gland mucus transferred by the dart are likely

to influence the degree of the putative effect(s) on the recipient. Signal variability correlating with signaler viability/quality is a requirement for the indicator/good genes mechanism of female choice, discussed below.

#### EXISTING HYPOTHESES FOR *HELIX* DART FUNCTION

Although not demonstrated empirically, dart shooting clearly extracts substantial material, energetic, and other costs (e.g., developmental; see the section below "Female Choice Based on the Love Dart, Relevant Aspects of the Reproductive Biology of *Helix*"). What possible benefit could the dart bring which might offset these costs? Existing hypotheses for dart function are of three types (Chung, 1987): reproductive isolation, sexual behavior coordination/stimulation, and sexual selection/conflict.

#### Reproductive Isolation Hypotheses

Some early workers (Diver, 1940; Webb, 1952) proposed that dart systems evolved and/or currently function to prevent interspecific matings. For many years, until the 1970s, the literature abounded with such explanations for species-specific sexual displays. However, there are several reasons for doubting the validity that this function is the primary one, both for the darts of *Helix*, in particular, and for sexual signals in general (Zahavi & Zahavi, 1997).

First, it is illogical that such a costly feature as dart shooting, performed late in the mating sequence, evolved expressly to prevent interspecific matings. If interspecific copulations result in low-fitness offspring and/or extract other fitness costs, selection would favor unambiguous, early, and relatively cost-free species recognition. In some sympatric *Helix* species, e.g., *H. aspersa* and *H. lucorum*, interspecific mating encounters have never been reported, even though sexually active individuals of both species were kept together for months or years (F. Giusti, personal communication; personal observation). The scarcity of reported helicid interspecific mating attempts suggests that species recognition occurs soon after initial contact between snails.

The hypothesis also predicts that, in order that the dart be used for species discrimination, its associated behavioral, physiological, and/or morphological parameters should be species-specific. The gross similarity of dart use *among* species and the wide variation in dart-shooting parameters *within* species are contrary to the species-discrimination hypothesis. Perhaps the species-specificity of dart shooting lies in the morphology of the dart, or in the composition of the dart mucus? Dart morphologies differ among species (Tomba, 1980), but it is doubtful that snails can "perceive" the shape of received darts, especially given the variability in dart shooting/receipt. Regarding the possibility that the dart mucus is species-specific, the only relevant result is Chung's (1986a) finding

that the helicine helixid *Cepaea nemoralis* exhibited GPR swelling and eversion when injected with *H. aspersa* mucus. The *C. nemoralis* response was qualitatively identical to *H. aspersa*'s response to injection of *H. aspersa* digitiform gland extract. It is therefore unlikely that the digitiform gland mucus acts as a species recognition cue.

Third, the hypothesis predicts that interspecific mating encounters should proceed readily up to the point of dart shooting, immediately after which the pair should break off their interaction. Reports of interspecific sexual encounters in helicids are rare, so it is difficult to find evidence either supporting or refuting this point. Beaumont (1988) reported that *C. nemoralis* and *C. hortensis* engaged in interspecific pairings in dense mixed-species experiments. The interactions were not observed carefully enough, however, to determine whether dart shooting influenced copulations. Petersen (1971) reported a single case of interspecific sexual behavior in which both partners (*C. nemoralis* and *C. hortensis*) passed through dart shooting. Only the *C. hortensis* shot a dart, but this did not strike the *C. nemoralis*; this latter individual exhibited dart-shooting behavior, but no dart was observed. Both snails attempted intromission but the pair failed to copulate. In this well described interspecific mating attempt, the failure to copulate appeared to result not from receiving the incorrect dart, but rather from incompatible copulation behavior. The two partners did not terminate their interaction at the critical stage of dart shooting. Finally, Webb (1951) observed an instance of courtship between two species of *Helminthoglypta* (Helminthoglyptidae). The individual of the larger species shot a dart into the smaller partner, which was gravely injured by the dart receipt. The courtship did not proceed, and the smaller, darted individual died 4 days later. The seriousness of the injury caused by the dart in this case renders Webb's observation difficult to interpret with regard to the dart-as-reproductive-isolation-mechanism hypothesis. Tompa (1980) reported that there are no other recorded observations suggesting that potential interspecific matings are inhibited by the dart.

The logic and evidence reviewed above indicate that the dart-as-reproductive-isolation-mechanism hypothesis should not be considered as a viable adaptive explanation of dart shooting.

### Sexual Behavior Stimulation/Coordination Hypotheses

The second hypothesis proposes that dart shooting stimulates the recipient sexually, facilitating temporal coordination between the partners and thereby increasing the likelihood that they achieve copulation. For many years, partner stimulation and/or coordination was a primary explanation for the function of precopulatory/courtship behavior in general (Bastock, 1967). Evidence specific to *Helix* consists of observations of the immediate

effects of dart shooting and receipt, or of injection of digitiform gland mucus, on sexually active snails.

A common idea has been that the digitiform gland mucus transferred by the dart contains a bioactive substance that stimulates the recipient. Dorello (1925) injected digitiform gland mucus into *H. aspersa* and found that the treatment elicited body wall musculature contractions and stimulated the nervous and reproductive systems. Goddard (1962) found in *H. aspersa* that the injury to snails caused by dissection induced activity of the penis, and proposed that dart penetration would have the same effect. He reported no effect of injection of digitiform gland mucus on the penis, concluding that the penis activity was induced by the physical trauma of dart receipt. Injection of digitiform gland extract by Börnchen (1967) in *H. pomatia* increased the frequency and amplitude of heart contractions. Chung's (1986a) injection of *H. aspersa* with digitiform gland extract caused eversions of the genital atrium and/or penis, in agreement with Dorello (1925). His conclusion was that "the dart may be used for traumatic inoculation of the mating partner with a contact pheromone that enhances sexual receptivity."

Adamo & Chase (1988) compared the durations of sexual stages in dart recipients and non-recipients of *H. aspersa*. The only difference found was the time between the first and second dart-shooting events: if the first dart hit and penetrated the recipient, the time to the second dart-shooting was significantly reduced by a mean of 26 min. No correlations were found between dart shooting/receipt and either the interval from the second dart shooting to successful copulation or the duration of copulation. The mean duration of the entire mating sequence was approx 490 min; the 26 min reduction thus represents a 5.5% time savings. Adamo & Chase (1990) subsequently showed, using dart sac- and/or digitiform gland-extirpated snails, that the decrease in courtship time was caused by the digitiform gland mucus, not by dart receipt alone: snail pairs that shot "dry" darts experienced matings that lasted as long as dartless pairings. Injection of digitiform gland extract into sexually active snails similarly produced a small but significant decrease in time-to-copulation, but only if the injection occurred when the snail was at an intermediate stage of sexual arousal (Adamo & Chase, 1990). Digitiform gland extract injection also inhibited locomotion and induced a temporary increase in the level of GPR eversion. Adamo & Chase (1990) concluded that the dart mucus contains a pheromone that reduces the time that the partners are in different, non-complementary stages. They speculated, but did not demonstrate, that this effect raises the likelihood that pairs copulate successfully.

The hypothesis that dart shooting functions to stimulate mutual sexual behavior, thereby increasing the probability of successful copulation, is contradicted in two thorough studies. Lind (1976) tested in *H. pomatia* whether dart receipt affected the partners' level of sexual activity, du-



ration of precopulatory behavior, and probability of successful copulation. By switching partners between two snail pairs (putting the first dart shooters together) he created pairs in which neither partner received a dart. Snails manipulated in this way mated normally. He found that such pairings resulted in significantly *shorter*, not longer, times-to-copulation; precopulatory behavior in pairs in which neither snail, one snail, and both snails received a dart lasted on average 3.0, 4.2, and 5.2 hr, respectively. He further reported that the post-dart-receipt behavior of a snail depended chiefly on that snail's activity level just prior to being shot: if the recipient was active before being shot, it remained active; if passive, it remained passive. Of those snails showing a change in activity upon dart receipt, twice as many became *less* active than more so. These observations led Lind (1976) to conclude that no facilitative effect of the dart on subsequent behavior of the recipient exists.

Lind's (1976) analysis was largely descriptive, although he did not refrain from commenting on the apparent non-adaptiveness of dart shooting. In contrast, Chung's (1987) study of sexual behavior in *H. aspersa* was undertaken deliberately "in order to document in detail the courtship of this snail and to determine whether dart receipt stimulates courtship or has another function." Chung (1987) carefully assayed the behavior of paired snails that either received a dart or not. As measures of sexual arousal he used the biting rate, the fraction of time spent out of mutual genital contact, and the rate of attempted copulation. He found no significant differences in the first two parameters between dart-receiving and non-dart-receiving snails. Additionally, in snails receiving a dart there were no significant differences before and after dart receipt in the biting rate or the fraction of time spent out of genital contact. Snails receiving a dart had significantly *lower*, not higher, rates of attempted copulation (the third parameter, above) than those not receiving a dart. In contrast to Adamo & Chase (1988, 1990), Chung (1987) did not find that the time between the first and second dart-shooting events was shorter if the first dart penetrated the recipient.

What can be concluded regarding the hypothesis that the dart functions to stimulate and/or coordinate snail sexual behaviors? That dart receipt has no demonstrable effect on the probability of copulation rules out a role for the dart in the overt stimulation of sexual behavior. (However, the effects of dart and/or digitiform gland mucus receipt may not be limited to those observable externally. As mentioned, Koene & Chase [1998b] found that digitiform gland mucus induced contractions of the female reproductive tract. The potential adaptive consequences of such "cryptic" effects of dart receipt are discussed below.) Coordination of sexual behavior by dart shooting may be admitted only if a perverse concept of coordination is applied: at the onset of precopulatory behavior, before the first dart-shooting event, the partners' behav-

iors are well synchronized; they become asynchronous and non-complementary upon the first dart shooting; and they are resynchronized only after the second dart shooting (Lind, 1976). Lind's (1976) and Chung's (1987) conclusions regarding the dart's effect in *H. pomatia* and *H. aspersa*, respectively, were that the dart does not function to synchronize or coordinate sexual behavior.

The observations of Giusti & Lepri (1980) and Giusti & Andreini (1988) of matings in *H. lucorum*, *H. aperta*, *Theba pisana*, *Tacheocampylaea tacheoides*, and *Eobania vermiculata* also fail to support the hypothesis that the dart coordinates/stimulates sexual behavior. These authors reported that copulation proceeded normally in the absence of dart receipt, although they did not quantify the times-to-copulation of dart-shooting vs. non-dart-shooting pairs. Giusti & Andreini (1988) hypothesized that dart shooting functions to test the partner's sexual receptivity or motivation. Assuming that biting and dart receipt represent adverse stimuli, Giusti and collaborators reasoned that only highly motivated snails proceed to copulate in the face of such noxious stimuli. Unmotivated snails would be identified and filtered out by dart shooting, leaving sexually motivated snails to concentrate their efforts on like individuals. There is no evidence to support this hypothesis. Once two snails pass through introductory behavior to the dart-shooting stage, the probability that they will proceed to copulation is quite high (approx. 95% in *H. aspersa*; personal observation), and does not depend on dart shooting/receipt. Also at odds with Giusti's hypothesis is the fact that, by shooting a dart at a given partner, the shooting snail forgoes the opportunity similarly to "test" other prospective partners for at least 7 days, the time required to manufacture a new dart. Other tactics to assess partner motivation, e.g., the extensive facial, oral, and genital contact occurring prior to dart shooting, would be far less costly to the snail doing the testing.

The evidence that dart shooting functions to stimulate the partner's sexual activity and/or to coordinate the snails' behaviors is at best equivocal. That dart shooting precedes copulation need not mean that its function is to facilitate the latter act.

### Sexual Conflict/Selection Hypotheses

Explanations of a different sort propose that the dart is an adaptation to resolve sexual conflict. These hypotheses generally assume that one of the sexual roles (male or female) is "preferred" over the other. That is, the biological constraints of a hermaphroditic species are such that the cost/benefit ratio of reproduction via one sexual mode is more favorable than that via the other, at least under certain conditions or at specific life history stages. Individuals will be selected to prefer to mate in the role offering the better cost/benefit tradeoff. If the same mode is preferred by all or most individuals, a conflict of in-

terest occurs between potential partners over the assumption of sexual roles (Charnov, 1979; Leonard, 1991, 1992). Conflicts of interest may arise between partners regarding the fates of gametes and zygotes and/or the amounts and kinds of resources invested in offspring by each partner. As paternal care is absent in pulmonates, the only conflicts of interest possible in this group are those regarding the extent of maternal provisioning and the fates of gametes.

Here I discuss two sexual conflict hypotheses as applied to animal hermaphroditic mating systems: Bateman's principle and certainty of parenthood. Bateman's (1948) principle states that the main factor constraining paternal fecundity is the male's ability to gain access to females and/or their eggs, whereas the corresponding limitation on maternal fecundity is the female's ability to convert resources into offspring. These constraints give rise to the situation in which potential individual fecundity via male function is greater than that via female function. Applied to hermaphrodites, Bateman's principle implies that individual hermaphrodites should prefer to reproduce via the male mode. Extending Bateman's logic, Charnov (1979) reasoned that simultaneous hermaphrodites pursue matings not so much to receive allosperm for fertilization (female function), but rather to obtain opportunities to inseminate partners (male function).

Chung (1987) first proposed that the *H. aspersa* dart serves to resolve the sexual conflict of interest between mating partners. Starting from the assumption that the male role is preferred, he hypothesized that male-acting snails shoot darts to coerce partners into accepting their (the shooter's) sperm and using it for fertilization. As one possible mode of dart-mediated partner coercion, Chung (1987) speculated that receipt of the dart and/or digitiform gland mucus might induce oviposition. However, Koene & Chase (1998a) reported no difference in oviposition rates or amounts between dart recipients and non-recipients (*H. aspersa*), and Baur & Baur (1992) found that precopulatory behavior, including dart shooting, did not increase oviposition in *A. arbustorum*. Other possible effects of dart receipt proposed by Chung (1987) are that the dart/mucus may serve to potentiate transferred sperm, inhibit sperm digestion, induce the displacement of previously stored sperm, suppress subsequent mating activity, or inhibit the storage of subsequently received sperm. He further speculated that "the dart may have evolved . . . in a kind of evolutionary escalation that allowed the evolution of increasingly larger or more effective darts to force the partner to act as a female" (Chung, 1987). Chung failed to consider, however, any possible evolutionary response by dart receivers to the coercive effects of the dart/mucus: if dart receipt entails a reproductive fitness cost, selection should favor adaptations to counter its effects.

Adamo & Chase (1996) restated Chung's (1987) hypothesis more strongly by proposing that dart shooters

manipulate their partners' reproductive physiology for their own (the shooter's) benefit. Adamo & Chase (1996) did not explicitly specify the fitness effect of receiving a shot dart, but by using the term "manipulate" they imply that the fitness of dart recipients is decreased. The same objection raised above regarding the evolution of resistance to the dart effect is applicable here, although Adamo & Chase (1996) speculated that the active ingredient in the dart mucus could be identical to a compound used by snails to control their own reproductive functions. If so, then any response evolved to combat the dart mucus effect might also interfere with a snail's ability to control its own female reproductive processes.

More recently, Koene & Chase (1998b) showed that the *in vitro* application of digitiform gland mucus to *H. aspersa* preparations induced muscular contractions and reconfigurations of the female genital tract. Specifically, the mucus acted to constrict the entrance to the bursa copulatrix (the sperm digestive organ) and to elicit peristalsis in the bursa diverticulum to pull in the received spermatophore. Both of these influences probably affect the fate of received sperm. Koene & Chase (1998b) interpreted their result in accordance with Chung's (1987) and Adamo & Chase's (1996) explanation for the dart: dart shooting is an adaptation by which males manipulate females to maximize their own (male) reproductive success. Koene & Chase (1998b) concluded, however, that the reason why males attempt to manipulate females via the dart derives from sperm competition. That is, the evolutionary rationale for dart shooting and for analogous male manipulative efforts is that they allow males to compete successfully against rival males.

Another concept relevant to sexual conflict and preferred sexual roles is "certainty of parenthood" (Leonard, 1991, 1992). In most mating systems, the certainties of parenthood of the female and male are likely to be unequal; the sex controlling fertilization has greater certainty that the investment committed will result in offspring. For example, in promiscuously mating species with internal fertilization, a mated female can ensure that all of the eggs she produces will both be fertilized and be her own, whereas a male is less certain that his mate's offspring will be fathered by him. Leonard (1992) proposed that the female role would be preferred in land snails due to that sex's greater certainty of parenthood. She hypothesized that the dart functions as an honest signal that the shooter will perform the less-preferred male sexual role (to donate sperm without guarantee of paternity) in order thereby to have the opportunity to reproduce in the preferred female role (to receive sperm and be guaranteed of maternity). Several predictions pertinent to the mating system of *H. aspersa* follow from the hypothesis, some of which were addressed by Adamo & Chase (1996).

The first prediction of Leonard's (1992) hypothesis as applied to *H. aspersa* is that non-dart-shooting individuals should be unwilling or unable to perform the male



role, i.e., to donate sperm to their partners. Contrary to this prediction, Adamo & Chase (1988) reported that all snails achieving successful intromission transferred a spermatophore ( $n = 70$ ), whether they had shot a dart or not. Similarly, Baur et al. (1998) reported all copulants (*A. arbustorum*,  $n = 92$ ) transferred spermatophores, 91 of which (99%) contained sperm.

Another prediction (Leonard, 1992) is that non-dart-shooting partners are unattractive as female mates because a snail's non-receipt of a dart might imply that its partner has mated within the last week (and thus recently received allosperm) or is a virgin. The first difficulty with this prediction is that it is not obvious why virgin snails would make poor female mates, as there is no evidence that reproductive abilities are affected by prior experience. Virgin snails may in fact be *more* attractive mates, as they lack previously stored allosperm and are less likely to carry venereally transmitted parasites. Second, non-receipt of a dart need not mean that the partner did not shoot a dart; the partner could have shot but missed. Dart non-receipt is thus not a reliable indicator of a partner's recent mating history. Further, snails which have recently received sperm may nonetheless be desirable as female mates, as subsequently received sperm can still be stored and used for fertilization. Baur (1994) found in *A. arbustorum* that the second (last) male to mate sired a mean of 32% of offspring; the range was 0–100%. Finally, if non-dart-shooting partners make poor female mates, one would expect snails not to copulate unless a dart had been received. This prediction is refuted by Lind (1976), Giusti & Lepri (1980), and Chung (1987).

A third prediction (Leonard, 1992) is that snails should refuse to intromit and/or to transfer sperm unless the partner reciprocates. That is, snails should decline to copulate as males only. It is true that unilateral intromissions are generally disallowed; the question is whether these are prevented from the male or female side. Rarely, one of a pair of snails succeeds briefly in achieving unilateral intromission. In these cases, both Giusti & Lepri (1980) and Chung (1987) reported that the intromitted snail appeared to expel its partner's unilaterally intromitted penis. Chung (1987) observed in these cases that the intromitting (male-acting) snail assumed the typical copulatory, i.e., quiescent, posture. The female-acting snail, in contrast, attempted actively to pull away and bite the partner's penis until it was withdrawn. It thus appears that a snail will consent to unilateral intromission only if it is the "intromitter," but will not allow unilateral intromission if it is the "intromittee." This is exactly the opposite of what Leonard's (1992) hypothesis predicts.

A theoretical deficiency of Leonard's hypothesis is that there appears to be no functional or mechanistic reason why dart shooting should serve to signal reliably that a snail will perform the less-preferred male role of delivering sperm. What prevents a snail from shooting a dart and then not transferring sperm?

In sum, the hypothesis (Leonard, 1992) that dart shooting in *Helix* is an adaptation to resolve the sexual conflict between snail partners preferring to mate as females is logically tenuous, and its empirical predictions appear to be refuted by the available evidence (Adamo & Chase, 1996).

## FEMALE CHOICE BASED ON THE LOVE DART

If the critiques given above are deemed acceptable, then only two hypotheses for the adaptive, ultimate function of dart shooting remain viable. (Proximally, dart receipt probably influences allosperm storage and digestion; Koene & Chase, 1998b.) One is that dart shooting is a male manipulative adaptation by which males compete with rival males by influencing their partners' usage of allosperm (Adamo & Chase, 1996). The other is that the dart is a male sexual signal used by females to select sperm received from different mates, i.e., females choose the fathers of their offspring based on their mates' relative dart shooting abilities (Charnov, 1979). The two hypotheses need not be mutually exclusive (Leonard, 1991). In this section I first review the logic of male manipulation and subsequently further develop the female choice hypothesis for dart function. I conclude by discussing known aspects of snail biology, as well as further experimental work, that may provide clues regarding the relative validity of these two hypotheses.

## Male Manipulation

Chung (1987), Adamo & Chase (1996), and Koene & Chase (1998b) hypothesized that *H. aspersa* individuals shoot darts in order to manipulate their partners into preferentially using their sperm for fertilization. This sort of manipulation is adaptive only if females mate promiscuously, and is therefore fundamentally a manifestation of male-male competition over access to females' eggs. If females were to mate with only a single male, then the reproductive interests of the two partners would be identical—both partners would achieve maximum reproductive fitness via the female's use of the male's sperm to fertilize all of her eggs—and the reproductive interests of the male would not be served by his attempting further to alter the female's use of his sperm. It is only when females receive sperm from more than one male that the interests of male and female partners come into conflict; one consequence of this is that males would then be selected to compete vicariously with rival males within the arena of the female (Eberhard, 1996). Selection on males to outcompete rivals for fertilization opportunities leads to the evolution of a class of manipulative strategies for biasing female reproduction, e.g., mate guarding, removing rival sperm, inducing oviposition, and influencing females' sperm handling (Andersson, 1994). In *H. aspersa*, the effects of the dart mucus on the female reproductive tract (Koene & Chase, 1998b), leading to the preferential

storage and/or use of transferred sperm, may represent such an apparently manipulative strategy derived from male-male competition.

Although the aim of male manipulative efforts is not explicitly to harm females, but rather to outcompete rival males, the influences listed above are not necessarily in the health and/or fitness interests of females. For example, copulating male *Drosophila* transfer to females seminal fluid compounds that incapacitate previously stored sperm, thereby providing the last-mating male with a sperm competition advantage (Harshman & Prout, 1994). The effects of these compounds on females are not entirely benign; females receiving more seminal fluid products suffered an increased death rate (Chapman et al., 1995). Chapman et al. (1998) found that mating reduces survival in females of 10 of 29 insect species from five of nine families. While the data do not represent an overwhelming trend, the positive cases indicate that receiving courtship and/or copulation exacts physiological costs, at least in some species. Further, Chapman et al.'s (1998) own results on the fly *Ceratitis capitata* point to independent survival costs derived from copulation (including the receipt of courtship) and from egg production. That is, the simple act of engaging in copulation and in-copula courtship comprises a cost of mating distinct from that of egg production. Additional examples of male strategies to influence female reproduction, and their effects on female physiology and fitness, are given by Eberhard (1996) and specifically in hermaphrodites in Michiels (1998).

It is important to distinguish between health/survival costs and total lifetime fitness costs; demonstration of the former does not automatically implicate the latter. In *Drosophila*, in which physiological costs of mating to females have been observed (Fowler & Partridge, 1989; Chapman et al., 1995; Chapman & Partridge, 1996), females allowed to choose their mates produced offspring with greater viability than did females denied a choice of mates (Partridge, 1980). Thus, even though females' receipt of courtship and mating can damage their health, they can nonetheless reap a fitness benefit by using courtship to assess and choose mates. This latter result was confirmed in *Drosophila* by Hoikkala et al. (1998), who additionally showed that specific components of the male sexual signal correlate with viability. Results demonstrating female benefits of choice based on receipt of male courtship have also been reported in other species (Welch et al., 1998; Alatalo et al., 1998). (Studies giving examples in which no significant indirect benefits appear to be derived from mate choice also exist; Alatalo et al., 1998.) The coexistence of these apparently paradoxical consequences of female receipt and assessment of male courtship, i.e., that they exact proximate costs but bring ultimate benefits to females, probably represents a general phenomenon.

Can male manipulation alone, arising from male-male competition, explain courtship behavior? A potential dis-

tinction between male manipulation and female choice hypotheses of dart function is that females should reap a net fitness benefit if they are using the information contained in the courtship signal as a basis for mate choice, whereas they are unlikely to benefit if they are the objects of manipulation only. Further, if male manipulation has detrimental health effects on females, as in *Drosophila* (Partridge, 1980) and *Ceratitis* (Chapman et al., 1998), but females nonetheless gain net fitness from receiving and utilizing sexual signals, then the evolution of courtship must be driven at least in part by female choice.

### Female Choice in *Helix*: Runaway vs. Indicator Mechanisms

Male manipulation does not consider the potential adaptive use by females of information contained in male courtship behaviors. The use of this information to inform mate choice may be selected if it allows choosy individuals to bear offspring in higher number and/or of higher quality. In many taxa, including *Helix*, males provide no direct benefits to their mates; female choice in these species is therefore unlikely to offer benefits via augmented fecundity. Instead, in species in which males provide nothing but sperm, female choice may bring indirect (genetic) benefits only.

Charnov (1979) hypothesized that the dart is a male sexual signal coevolved with a female preference for dart shooting via a fisherian runaway process. In runaway, the genes for the male signal trait and for the female preference become linked through assortative mating between males expressing the signal, and females expressing the preference for it (Andersson, 1994). To get started, the process requires that females initially prefer males bearing a particular perceivable trait. The specific origin of the pre-existing female preference is irrelevant; for example, it could be inherent in the species' sensory system (Ryan, 1990). Although the initial source need not be specified in order to propose subsequent trait-preference coevolution by runaway, Charnov (1979) nevertheless gave two hypotheses to explain why females might initially have preferred ancestral dart shooters: the dart (or its forebear) was a nuptial gift of calcium, and/or it demonstrated an increased ability to metabolize that resource.

Once a runaway process is underway, it no longer relies upon whatever correlation may have formerly existed between the male signal trait and other traits; the genetic linkage between the male signal trait and the female preference is alone sufficient to maintain their subsequent coevolution. The signal trait is therefore likely to come to be arbitrary with respect to other male characters. Charnov clearly cited the runaway process itself as the mechanism by which dart shooting evolved and is maintained; the hypothesized correlations between the dart and other male qualities were simply his guesses as to how the process began (Charnov, 1979:2483): "One wonders if the

love darts of some snails are the result of such a (runaway) process.”

Twenty years passed before experimental studies were undertaken to test Charnov's hypothesis regarding dart-based female choice in snails. Koene & Chase (1998a) refuted the idea that the dart is a nuptial gift of calcium: the dart contains too little of that element relative to the amount in a clutch of eggs, and it is only rarely absorbed by the recipient. Further, J. Koene (unpublished) found that the calcium in absorbed allodarts is excreted. The hypothesis given here for the dart's adaptive function picks up on Charnov's second notion, that dart shooting is a “demonstration . . . of increased ability” (Charnov, 1979:2483). Charnov did not develop this idea into a distinct hypothesis for dart shooting nor as an explanation for the evolution of sexual signals in general. (Subsequent authors have done the latter; Andersson, 1994.) At the time, Zahavi's (1975, 1977) “handicap principle,” also known as the “indicator” and “good genes” hypothesis when applied to sexual selection, had just been published as an explanation for the evolution of female preferences and male sexual signals. As individual reproductive fitness is influenced in part by offspring quality, there is selective pressure on individuals to maximize the genetic quality of their offspring via mate choice. Applied to sexual selection, the handicap principle states that honest signaling systems evolve in response to selection for the identification of high genetic quality mates: as a result of pressure on individuals to secure mates with good genes, preferences evolve for traits that correlate with, and therefore indicate, the genetic quality of potential mates. The ideal preferred trait is that whose magnitude varies perceivably and reliably with mate quality; these preferred indicator traits can coevolve with the preference to become sexual signals. (The handicap principle may be applied to other communication systems, such as between predators and prey. I use “indicator,” as have others [e.g., Andersson, 1994], to refer to a signaling mechanism in which a “handicap” trait conveys information about the genetic quality of potential mates.)

Population geneticists were slow to accept the operation of the indicator mechanism (e.g., Bell, 1978), and perhaps Charnov (1979) considered it to be an unlikely explanation for dart shooting. Regardless, the runaway and indicator hypotheses are now thought to be the best explanations for the evolution of preferences for sexual signals (Pomiankowski, 1988; Andersson, 1994; Andersson & Iwasa, 1996).

The hypothesis presented here is that dart shooting in *Helix* evolved as a male sexual signal used by females as an indicator of mate viability. Females choose the fathers of their offspring by selecting which received allosperm to use for fertilization of their eggs based on their assessment of their mates' sexual signal—dart shooting effectiveness. Charnov (1979) explained the dart by the runaway process; I invoke the indicator mechanism. A

crucial consequence of female choice by the indicator mechanism, as opposed to runaway, is that the positive correlation between magnitude of expression of the signal trait and mate quality provided by the indicator mechanism allows females to produce offspring of above-average viability. If population viability is sub-maximal and if viability is heritable, females gain a distinct fitness benefit via the indicator mechanism. (An additional consequence, relevant to species selection, is that sexual selection by female choice by the indicator mechanism potentially increases population viability above the level achieved by natural selection alone.) In contrast, in runaway the relationship between the signal trait and mate quality is arbitrary, and so, on its own, sexual selection by the runaway process does not generate increased offspring fitness, relative to natural selection alone.

Gaining indirect fitness benefits via female choice appears simple in principle, but a practical difficulty arises for females: how to identify high-viability mates? Females will generally not be able to evaluate mate viability directly, casually. Instead, they must “search” for a perceivable male trait whose magnitude correlates with viability. (“Perception” does not require consciousness, nor even nervous system involvement.) Preferences for specific traits may arise as a consequence of sensory biases (Ryan, 1990) or other attributes of a species's biology. Of the many possible traits expressed by males and preferred by females, the expression of one or some of these traits may be correlated with viability such that high-viability males tend to express the trait better. Other male traits will bear no such correlation, or a weaker one, with viability. Females perceiving and preferring the male trait with the tightest correlation with male viability will consequently mate with that non-random sample of males having highest mean viability. If viability is sufficiently heritable and the fitness benefit to females outweighs the cost of choosing (Andersson, 1994), both female preference and male trait—sexual signal—will be selected.

Which male traits correlate best with mate viability, eventually evolving into male sexual signals? The unifying feature of such traits, regardless of a given species's biological constraints, is costliness. Precisely because signals are costly, higher-viability males can better support them than can lower-viability males. Critically, the proportional cost that a high-viability male supports by bearing a given signal is less than that borne by a low-viability male bearing the same-magnitude signal (Grafen, 1990a, b; Getty, 1998). The costliness of sexual signaling therefore ensures the correlation between a male's viability and his ability to signal. Such signals are thus *non-arbitrary*, in that they have evolved so as to be *indicators* of the bearer's viability (Zahavi & Zahavi, 1997).

#### Relevant Aspects of the Reproductive Biology of *Helix*

Male manipulation and female choice need not be mutually exclusive and can in fact be seen as two sides of

the same process. According to Eberhard (1998), we can think of females as setting the rules of the game and males as the more active players. Likewise, if female choice occurs in a given species, both the runaway and indicator/good genes mechanisms may be operating. Here I review some aspects of the biology of *Helix* which may support the female choice perspective of the evolution of the adaptive function of dart shooting.

(i) The gross anatomical and behavioral characteristics of *Helix* and some other helicids provide ample opportunities for mate choice. Courtship and promiscuity allow both the assessment of multiple mates and the receipt of sperm from those mates. The indiscriminate acceptance of sperm from all partners might be taken as evidence against mate choice. However, *Helix* and some other helicids have evolved elaborate anatomical adaptations that allow both the selective digestion and long-term storage of allosperm (Tompa, 1984; Baur, 1998). Of the sperm received from a given single copulation, only a very small portion is stored (Lind, 1973). It is unlikely that the quantities of sperm stored from each copulation are equal, and Koene & Chase's (1998b) finding that the dart mucus affects contractions of the female reproductive tract suggests that the amount of allosperm stored depends on dart receipt, which in turn depends on the partner's dart shooting ability. If an individual's dart shooting ability correlates with its viability, then female choice in *Helix* may be manifested by a snail's ability to control the amount of allosperm stored from each copulation based on its perception of its mate's dart shooting. This perception need not be "conscious," as the regulation of sperm storage by dart/mucus receipt could be mediated by a simple chemosensory/endocrine pathway. Alternatively, individuals may exercise female choice by selecting which stored sperm (of those received from different mates) are used for fertilization of eggs. Such a selective sperm retrieval mechanism would require both the separate storage of sperm from different mates and a memory of whose sperm is in which spermathecal sac; there is no evidence for either of these phenomena. By whatever mechanism, mate choice by sperm selection in *Helix* and other species with similar mating systems (Michiels, 1998) may occur cryptically after copulation but be based on information received during courtship before, during, and/or after copulation (Eberhard, 1996, 1998).

(ii) Can individual snails reap indirect benefits from female choice of mates? The resolution of this issue relies largely on estimates of genetic variation and heritability of viability traits. Potential sources of genetic variation include parasite-host coevolution (Hamilton & Zuk, 1982), immigration of individuals adapted to different local conditions (Slatkin, 1978), and mutation (Kondrashov, 1988). Although all of these factors likely maintain the genetic variance and heritability of viability traits above zero, the question remains whether the magnitudes of these two parameters in natural populations are sufficient

to make mate choice worthwhile. Multiple studies (references in Dupont-Nivet et al., 1997) on genetic variation and heritability in *H. aspersa* indicate that, for example, shell size is heritable (shell dimension heritabilities of 0.2–0.8 are cited). Dupont-Nivet et al. (1997) found heritabilities of approx. 0.4 for both shell size and body weight, two traits that likely affect viability. What is lacking is a rigorous population genetic analysis determining whether the heritability values found empirically are in fact sufficient to allow mate choice to be adaptive. In addition to the measured heritabilities, a proper analysis would require extensive data regarding the species's life history, mating system, mutation rates, parasites, etc. In the absence of such a study, it nevertheless seems reasonable to propose that snails do indeed have something to gain from mate choice.

(iii) Relevant to the heritability issue is the fact that native populations of *Helix* and some other helicids harbor a multitude of parasites. "Arms races" in which evolving parasite adaptations continuously exert pressure selecting for host counteradaptations are likely to boost genetic variation and heritability of viability traits (Hamilton & Zuk, 1982). Of additional interest is the fact that many parasites of helicids inhabit the host's reproductive tract and/or dart-associated organs themselves; parasite transfer is venereal, and parasitism may directly affect a snail's ability to generate the sexual signal.

(iv) Both the runaway and indicator hypotheses for courtship signal-mediated female choice require that signal magnitude vary among males within the population. This is clearly the case for *Helix* and some other helicids; although the courtship appears stereotyped, there is substantial variability in dart shooting effectiveness and other parameters. This variability has gone unappreciated, perhaps because of the presumed role for the dart in facilitating copulation. In fact, the spectrum of dart shooting effectiveness ranges from none at all to sudden, well-aimed, and forceful dart ejection. Additionally, I have observed many cases of apparent misfirings, including partial and/or premature dart shootings and "self shootings" (a single self-inflicted darting was observed in approx. 150 pairings); these misfirings may represent inferior signals by low-viability individuals. Variability in the quantity and quality of dart mucus produced would contribute further to total signal variability.

(v) Related to this aspect is whether the dart is a "costly" signal. In terms of the materials involved the dart cannot be said to be expensive. The amount of calcium in a dart is equivalent to that in a single egg (Koene & Chase, 1998a), which represents less than 1% of a typical season's production. It is conceivable that the mucus transferred by the dart contains a substance that is costly to produce or acquire, but the composition of the mucus is unknown. However, although there is no evidence that the dart and mucus are materially expensive, dart shooting as a complete behavioral act may be quite costly as

measured in other currencies. The total cost of dart shooting consists of the efficient "presentation" of the signal to the intended receiver. This cost of presentation includes the proximate energetic costs of dart shooting, the genomic/information costs of encoding a properly functioning dart system (revealed through congenital defects in dart system function), and the metabolic/anatomical costs of building and maintaining the dart and mucus delivery system. None of these has been estimated in terms either of energy or fitness units, but the wide variation in dart-shooting ability and the not inconsiderable mass and complexity of the associated organs are consistent with the notion that dart-shooting is a costly signal (Leonard, 1991).

### Questions and Further Research

Many of the gaps in our knowledge of the biology of *Helix* are directly relevant to the hypotheses for dart function discussed here. For example, the indicator hypothesis for female choice based on male sexual signals requires a specific relationship between signal magnitude and signaler viability (Grafen, 1990a, Getty, 1998): high-viability males should produce a higher-magnitude signal than low viability males. This question has not been addressed in *Helix*. The answers to a second set of questions, whether greater dart-shooting effectiveness (signal magnitude) results in greater potential or actual paternal reproductive success, have recently been published. Both the amount of sperm stored by mates (Rogers & Chase, 2001) and paternal reproductive success (Landolfi et al., 2001) have been shown to depend on dart shooting effectiveness. However, the indicator, runaway, and male manipulation hypotheses for dart function all predict that better-shooting snails will experience higher levels of sperm storage and paternal reproductive success. Demonstrations of correlations between these parameters will therefore not distinguish among these more ultimate hypotheses for dart function. A third question is that of the bioactive agent in the dart (digitiform gland) mucus. If the mucus is responsible for the differences in allosperm storage and paternal reproductive success, as Koene & Chase's (1998b) study implies, then a study of its composition would be very useful indeed.

Regarding female choice and male manipulation hypotheses for dart shooting, clarification would be aided by the resolution of whether females benefit from receiving courtship, i.e., from dart receipt. The putative fitness benefit would derive from female assessment of the male sexual signal, allowing female choice of mates. If females do derive a fitness benefit from dart receipt, can they be regarded as being "manipulated" by males? On the other hand, the female choice hypotheses would be refuted if it were shown that females suffer a net reproductive fitness decrement while males derive benefits from dart shooting. It nonetheless seems possible that both pro-

cesses, female choice and male manipulation, have interacted to influence the evolution of courtship behaviors and mating systems (Eberhard, 1998). (Manipulation may be more significant in reciprocal hermaphrodites than in gonochorists because the former always participate in courtship simultaneously as both males and females. Selection for increasing male mating opportunities necessarily "exposes" simultaneous hermaphrodites to higher rates of courtship and mating as a female. In contrast, female gonochorists may better optimize costs and benefits of courtship and mating for that sexual mode.)

The theoretical feasibility of the operation of female choice raises a further question: whether this mate choice is sustained by fisherian runaway and/or indicator mechanisms. If signal cost scales with signal magnitude but yet is proportionally lower for high-viability than for low-viability males, it may serve as an indicator of phenotypic viability/genotypic quality. Alternatively, runaway does not specify any consistent relationship between an individual's ability to signal and any of its other qualities (besides its ability to attract mates). Because the runaway and indicator processes likely operate synergistically toward the same end, rendering their distinction by empirical methods has proven to be a challenge.

The resolution of these general issues is central to a full understanding of animal courtship, sexual selection, and the evolution of mating systems.

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## Identical Carbonic Anhydrase Contributes to Nacreous or Prismatic Layer Formation in *Pinctada fucata* (Mollusca: Bivalvia)

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**Abstract.** We have found a carbonic anhydrase (CA) in the prismatic layer of *Pinctada fucata*. This CA has the same kinetic properties as Nacrein, which is a CA existing in the nacreous layer of *Pinctada fucata*. We have examined the effects of inhibitors on the enzyme activity. Sodium sulfide and sulfanilamide are typical inhibitors of various types of CA; however, a CA in the prismatic layer and Nacrein were found to be resistant to sodium sulfide and to show a weak resistance to sulfanilamide. This is the first report of a carbonic anhydrase with resistance to sodium sulfide. The molecular mass of the prismatic layer CA was estimated by SDS-PAGE to be approximately 60 kDa. Moreover, we have determined the N-terminal amino acid sequence of a CA in the prismatic layer. The sequence of the first 11 amino acids was in agreement with that of Nacrein, as deduced from the cDNA sequence. From these results, we have concluded that the carbonic anhydrase of the prismatic layer is Nacrein. Nacrein contributes to the formation of a prismatic layer as well as a nacreous layer of mollusk shells as a carbonic anhydrase and is a matrix component.

### INTRODUCTION

Calcite, aragonite, and vaterite are crystal polymorphisms of calcium carbonate in biomineralization (Lowenstam, 1981; Lowenstam & Weiner, 1989). Of these, calcite is the most stable, and vaterite is the most unstable. Aragonite is slightly less stable than calcite at ambient temperature, but is widespread in marine organisms. Mollusk shells are composed of aragonite and/or calcite, and the organic matrix comprises 0.01–5% by weight of the shells. In the case of the pearl oyster *Pinctada fucata* (Gould, 1850), the outer prismatic layer contains calcite, and the inner nacreous layer contains aragonite. These layers contain organic matrix secreted by the mantle epithelia. The organic matrix consists of EDTA-soluble and insoluble proteins (Hare, 1963; Watabe, 1984; Mann, 1988). The formation of the two types of crystal is regulated by the matrix protein constituents. Some of them play an important role in the chemical control of crystal polymorphisms (Belcher et al., 1996; Falini et al., 1996; Samata et al., 1999).

It has been suggested that carbonic anhydrases (CA) that catalyze the interconversion of  $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{H}^+$  participate in the process of calcification (Benesch, 1984; Kakei & Nakahara, 1996) and mollusk shell formation (Wilbur & Jodrey, 1955; Freeman, 1960; Medakovic & Lucu, 1994). It is believed that the CA of mantle

epithelium facilitates the secretion of  $\text{HCO}_3^-$  for this calcification (Boer & Witteveen, 1980). We have already shown that a 60 kDa protein called Nacrein, which exhibits CA activity, exists in the EDTA-extract of the nacreous layer of oyster pearls (Miyamoto et al., 1996). Nacrein is an important factor in calcium carbonate crystallization, acting as a structural protein and a catalyst that provides the carbonate ion. We predicted that a Nacrein-like protein also participates in formation of the prismatic layer. Based on the results of the present study, we have now identified and characterized a CA in the EDTA-extract of the prismatic layer of *Pinctada fucata*. Here we report the biochemical properties of prismatic layer CA and discuss the function of CA in biomineralization.

### MATERIALS AND METHODS

#### Isolation of EDTA-Soluble Proteins

The prismatic layer was separated by cutting the shell edges with scissors. After the removal of epiphytes, the shell was crushed to a fine powder. The powdered shell (20 g) was extracted with 100 mL of 0.5 M EDTA (pH 8.0) with continuous stirring for 3 days at room temperature. The EDTA-soluble fraction was isolated from the insoluble matrix by centrifugation at  $\times 30,000$  g for 20 min. The supernatant (80 mL) was dialyzed against 3 liters of  $\text{H}_2\text{O}$  with three changes. The dialyzed fraction (300 mL) was lyophilized and then dissolved in 10 mL of 10 mM Tris-HCl (pH 8.0). The sample was dialyzed against 10 mM Tris-HCl (pH 8.0), followed by concentrated. Preparation of the EDTA-soluble extract of the nacreous layer of *Pinctada fucata* is the same as de-

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scribed above. The amount of protein was determined by using Protein Assay Dye Reagent (Bio-Rad).

### Carbonic Anhydrase (CA) Assay

The assay of carbonic anhydrase activity was performed as described by Miyamoto et al. (1996). Six drops of phenol red, 3 mL of 20 mM Veronal buffer (pH 8.3), and 20–200  $\mu$ L of the test-material-containing solution were mixed and placed in ice water. The reaction was started by the addition of 2 mL of ice-cold water saturated with  $\text{CO}_2$ , and then the time for the pH to drop to 7.3 was measured. Definition of units is as follows: unit =  $(T_0 - T)/T$ , where T and  $T_0$  are the reaction times required for the pH change from 8.3 to 7.3 at 0°C with and without a catalyst, respectively. Assay of enzyme activity in the presence of inhibitor was carried out as follows: all reagents in the assay mixture except the substrate were premixed in the reaction vessel for 10 min at 0°C. The reaction was started by the addition of the substrate.

### SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Proteins were subjected to sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis on 10% acrylamide gels, as described by Laemmli (1970).

### N-Terminal Amino Acid Determination

Proteins of the EDTA-soluble fraction were separated by 10% SDS-PAGE and were blotted onto a PVDF membrane (Millipore) using a dry blotting system (Nippon Eido). After Ponceau S staining, the band was cut out and then subjected to N-terminal amino acids sequence analysis.

### DEAE-Sephacel Column Chromatography

Approximately 20 mg of the soluble fraction of the prismatic layer was loaded onto a DEAE-Sephacel (Pharmacia) ion-exchange column ( $10 \times 1.5$  cm) equilibrated with 10 mM Tris-HCl (pH 8.0). After washing the column with 5 mL of 10 mM Tris-HCl (pH 8.0), the soluble fraction was chromatographed in the same buffer at a flow rate of 8 mL/h using a linear 0–0.8 M NaCl gradient. Fractions of 2 mL were collected, and carbonic anhydrase activity was assayed. The fractions containing carbonic anhydrase (Fraction Number 31–40) were pooled and then dialyzed for 10 mM Tris-HCl (pH 8.0), followed by concentration to 1 mL.

### Gel Filtration Chromatography

The concentrated sample was chromatographed over a Cellulofine GCL-300 sf (Seikagaku Kogo Co.) column ( $95 \times 1.5$  cm) equilibrated with 10 mM Tris-HCl (pH 8.0) containing 0.2 M NaCl at a flow rate of 12 mL/hr.

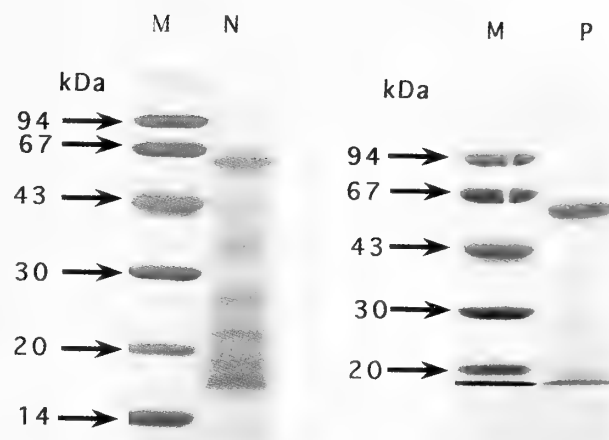


Figure 1. Comparison of SDS-PAGE Pattern of EDTA-soluble proteins extracted from the nacreous and prismatic layers of *Pinctada fucata*. 10  $\mu$ g of proteins were subjected to 10% SDS-PAGE. Lane N, EDTA-soluble proteins were extracted from the pearl nacreous layer. Lane P, EDTA-soluble proteins were extracted from the prismatic layer. Lane M, Protein markers (GIBCO BRL).

Fractions of 1.2 mL were collected, and carbonic anhydrase activity was assayed. Each fraction containing carbonic anhydrase was dialyzed separately for 10 mM Tris-HCl (8.0) followed by concentration to approximately 100  $\mu$ L. To examine the purity, 20  $\mu$ L of the concentrated fractions was subjected to 10% SDS-PAGE.

## RESULTS

The protein components of the EDTA-soluble fraction in the prismatic layer were compared with those of the nacreous layer. Proteins were loaded onto 10% SDS-PAGE. The molecular mass of the major protein was approximately 60 kDa in both layers (Figure 1). The 60 kDa protein of the nacreous layer is Nacrein (Miyamoto et al., 1996).

We assayed the carbonic anhydrase (CA) activity in the soluble fraction extracted from the prismatic layer. Bovine erythrocyte CA and Nacrein in the soluble fraction of the nacreous layer showed notable CA activity (Table 1). The soluble fraction extracted from the prismatic layer also contained CA activity. Although the specific activity was relatively lower than that of bovine erythrocyte CA, it was almost the same as that of Nacrein. The velocity of the enzyme reaction was increased 2 times by using a twofold amount of enzyme. A large amount of Bovine Serum Albumin (BSA), which has no enzyme activity, and a heat-inactivated soluble fraction showed no CA activity. These results indicate the presence of carbonic anhydrase in the soluble fraction extracted from the prismatic layer of *Pinctada fucata*.

We next examined the effects of inhibitors on the CA activity. Sodium sulfide and sulfanilamide are typical in-

Table 1

Assay of carbonic anhydrase activity of the EDTA-soluble fraction extracted from the prismatic layer of *Pinctada fucata*.

Sample	Amount used for assay (mg)	T (sec)	Specific activity (units/mg)
—	—	410 (= T <sub>0</sub> )	—
BSA <sup>1</sup>	6	430	—
BECA <sup>2</sup>	0.3	50	2.4 × 10 <sup>4</sup>
Soluble fraction (nacreous layer) (Nacrein)	3	40	3.3 × 10 <sup>3</sup>
Soluble fraction (prismatic layer)	1.5	100	2.1 × 10 <sup>3</sup>
Soluble fraction (prismatic layer)	3	50	2.1 × 10 <sup>3</sup>
Soluble fraction (prismatic layer) (heat-inactivated)	3	400	—

<sup>1</sup> BSA: Bovine serum albumin.<sup>2</sup> BECA: Bovine erythrocyte carbonic anhydrase.

hibitors of various types of carbonic anhydrases (Val, 1996). The activity of bovine erythrocyte CA was almost inhibited by these inhibitors (Table 2), as reported previously (Kiese & Hasting, 1940; Davenport, 1945). However, Nacrein was resistant to sodium sulfide and showed weak resistance to sulfanilamide. These results were identical to those for the prismatic layer CA.

To purify the carbonic anhydrase in the prismatic layer, we performed column chromatography. After DEAE-Sephacel column chromatography, the concentrated sample was passed through a Cellulofine GCL-300 sf column (Figure 2). Each fraction containing an enzyme activity was dialyzed separately for 10 mM Tris-HCl (8.0) and then concentrated to approximately 100 µL. To examine the purity, the concentrated fractions were applied to 10% SDS-PAGE. Fraction number 35 showed a single protein band that was almost homogeneous (Figure 3). The protein had an approximate molecular mass of 60 kDa. Fraction number 39 contained a larger amount of the 60 kDa protein than that of fraction 35 as well as a large amount of contamination which had an approximate molecular

mass of 40 kDa. However, the total enzyme activity of this fraction was approximately 1.7 times greater than that of fraction 35. From these results, we conclude that the approximate molecular mass of the prismatic layer CA is 60 kDa.

We have determined the N-terminal amino acid sequence of the 60 kDa protein. The sequence of the first 11 amino acids agreed with that of Nacrein as deduced from the cDNA sequence (Figure 4).

## DISCUSSION

Carbonic anhydrase (CA) is a ubiquitous enzyme existing in every tissue and cell type. Various isozymes of CA are now known (Tashian, 1989; Henry, 1996) and play an important role in acid-base balance, ion transport, maintenance of ionic concentration, and modulation of hemoglobin O<sub>2</sub> affinity (Cameron, 1979; Henry, 1984; Forster et al., 1986). CA also participates in biomineralization, and it is well known that CA is an essential enzyme of calcification (Wilbur & Jodrey, 1955; Freeman, 1960;

Table 2

Comparison of the effects of inhibitors on the activity of carbonic anhydrases. CA activity was expressed as a percentage of the activity in the absence of inhibitor.

Sample	Used amount for assay (mg)	Relative activity inhibitor		
		—	Sodium sulfide	Sulfanilamide
BECA*	1	100		
	1		11	
	1			2
Nacreous layer soluble fraction (Nacrein)	1.5	100		
	1.5		100	
	1.5			48
Prismatic layer soluble fraction	1.5	100		
	1.5		100	
	1.5			40

\* BECA: Bovine erythrocyte carbonic anhydrase.

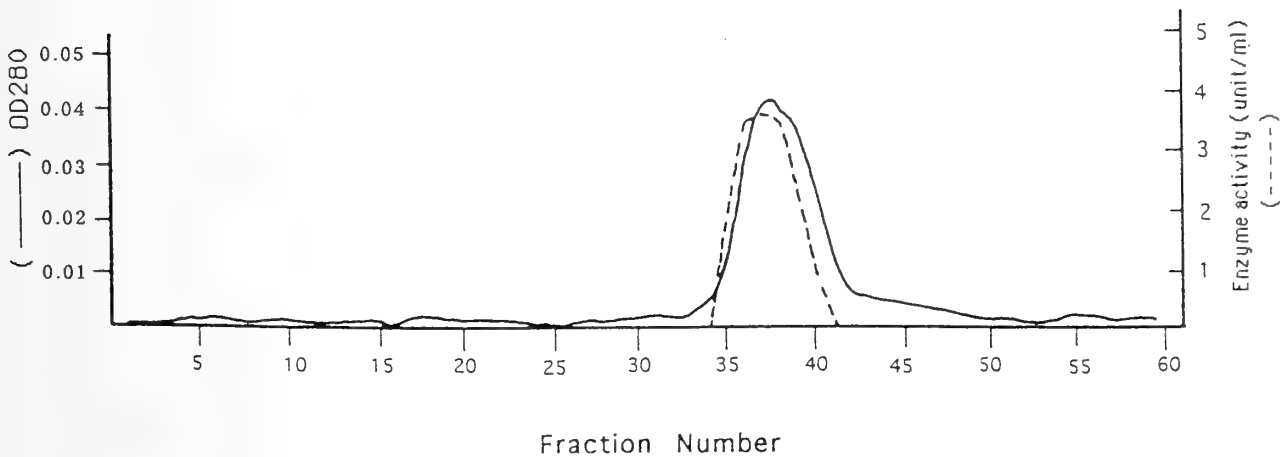


Figure 2. Cellulofine GCL-300 sf chromatographic profile of CA-containing fractions obtained from a DEAE-Sephacel column chromatography. Solid line, absorbance at 280 nm; dashed line, enzyme activity.

Bore & Witteveen, 1980). The mantle of the mollusk shell contains CA activity (Medakovic & Lucu, 1994; Freeman & Wilbur, 1948). We have recently identified the carbonic anhydrase named Nacrein in the EDTA-soluble matrix of the nacreous layer in oyster pearls and have isolated its cDNA (Miyamoto et al., 1996). Based on the amino acid sequence, Nacrein appears to contain two functional domains, one a carbonic anhydrase domain and the other a Gly-Xaa-Asn (Xaa = Asp, Asn, or Glu) repeat domain. It has been assumed that Nacrein contributes to the formation of  $\text{HCO}_3^-$  ions in calcification, and functions as a matrix component of aragonite crystal. The prismatic layer contains calcite, which is another polymorphism of  $\text{CaCO}_3$ , in addition to aragonite of the nacreous layer. We

therefore assumed that the prismatic layer contains a carbonic anhydrase that differs from Nacrein, and that this enzyme contributes to calcite formation.

We have identified in the present study a carbonic anhydrase in the extract of the prismatic layer of *Pinctada fucata*. The specific activity was found to be relatively lower than that of bovine erythrocyte CA and almost the same as that of Nacrein.

Sodium sulfide and sulfanilamide are well known inhibitors of CAII (Davis, 1959, 1961). The carbonic anhydrase in the prismatic layer was found to be resistant to sodium sulfides and to have weak resistance to sulfanilamide. These results are almost the same as those for Nacrein. This is the first report, however, of a carbonic anhydrase resistant to sodium sulfide. The mechanism of resistance to sodium sulfide is unknown.

To determine the molecular mass of carbonic anhydrase in the prismatic layer we further purified the protein

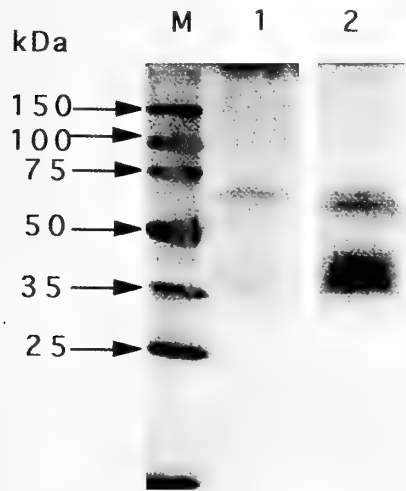


Figure 3. SDS-PAGE electrophoretic pattern of CA-containing fractions. Lane M, Perfect Protein<sup>®</sup> Markers (Novagen). Lanes 1 and 2 correspond to the fraction numbers of 35 and 39, respectively.

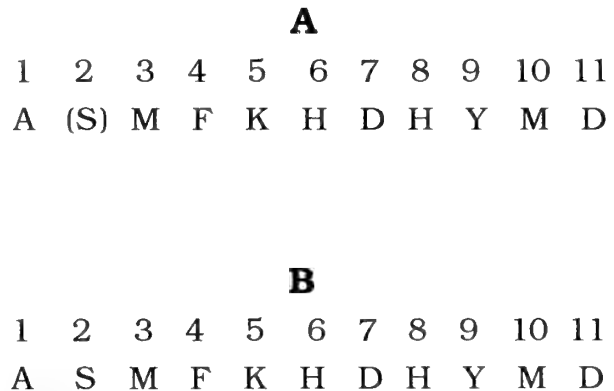


Figure 4. Sequence alignment of the N-terminal of the prismatic layer CA with Nacrein. A. Prismatic layer CA. B. Nacrein. The amino acid in parentheses is uncertain.

by means of Cellulofine GCL-300 sf gel filtration column chromatography. Subsequently, each fraction containing enzyme activity was subjected to SDS-PAGE. This analysis showed the presence of an approximately 60 kDa protein exhibiting enzyme activity. This molecular mass was equal to that of Nacrein.

To determine the amino terminal sequence, PVDF membrane transferred a 60 kDa protein was subjected to a sequence analyzer. The sequence of the first 11 amino acids agreed with that of Nacrein. Based on the results described above, we have concluded that the carbonic anhydrase of the prismatic layer is Nacrein. This result is unexpected,

Recently, the cDNA of a Nacrein-like protein called N66 was cloned from *Pinctada maxima*. RT-PCR analysis of the N66 mRNA revealed that this gene is transcribed in the dorsal region of the mantle, which is responsible for nacreous layer formation, and in the mantle edge, which is responsible for prismatic layer formation (Kono et al., 2000). These results are in agreement with the conclusions of the present report.

The presence of Nacrein in both the aragonite nacreous and calcite prismatic layers is suggestive with regard to the role of the Gly-Xaa-Asn repeat. We assume that this repeat is not related to the regulation of a crystal polymorphism of calcium carbonate. Recently, a nacreous layer-specific new matrix protein family was isolated from the EDTA-insoluble matrix of the nacreous layer of *Pinctada fucata*, and it was shown that this protein family designated N16 (N16-1,2,3) induces an aragonite crystalline layer (Samata et al., 1999). Based on its amino acid sequence, which is in agreement with N16-3 except that residue 58 is N, Pearlin also belongs to this family (Miyashita et al., 2000).

It seems likely that Nacrein is involved in the regulation of crystal growth and/or morphology via an interaction between the Gly-Xaa-Asn repeat and certain crystal faces or via coordination with another matrix protein(s). Soluble protein(s) that regulate calcite crystal growth or shape by means of an interaction with a calcite crystal surface are already known in shells (Walters et al., 1997) and sponge (Aizenberg et al., 1995). Biochemical characterization of these proteins, however, has not yet been carried out.

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## Thin Layer Chromatographic Analysis of Lutein and $\beta$ -carotene in *Biomphalaria glabrata* Maintained on a High Fat Diet

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**Abstract.** Thin layer chromatographic analysis was used to determine lutein and  $\beta$ -carotene in *Biomphalaria glabrata* snails maintained on a high fat diet of hen's egg yolk. The mean values of lutein in snails on a Romaine lettuce diet were approximately  $\times 3$  and  $\times 2.5$  at 14 and 20 days postculture, compared to those of snails maintained on the yolk diet. Likewise, the mean values of  $\beta$ -carotene for snails on the lettuce diet were approximately  $\times 1.5$  and  $\times 6.6$  at 14 and 20 days, respectively, compared to those from the snails on the yolk diet. The only significant differences in values (Student's t-test,  $P < 0.05$ ) was at day 20 at which time the mean percent of  $\beta$ -carotene in the snails on the high fat diet was significantly reduced compared to snails on the lettuce diet. The concentration of lutein in the lettuce was about  $\times 3.5$  that in the egg yolk. The concentration of  $\beta$ -carotene in the lettuce was  $\times 8$  that in the egg yolk. In general, the concentration of these lipophilic pigments in *B. glabrata* reflected the content of lutein and  $\beta$ -carotene in the lettuce and egg yolk diets.

### INTRODUCTION

Numerous studies have reported the use of a hen's egg yolk diet to observe nutrition in uninfected *Biomphalaria glabrata* (Say, 1816) and snails infected with larval schistosomes and echinostomes (see reviews in Fried & Sherma, 1990, 1993). These studies have observed mainly the effects of the egg yolk diet on the lipid content of the snails (Fried & Sherma, 1990, 1993), although a recent study by Kim et al. (2001) has examined the effects of this diet on the carbohydrate content of the snail. Because effects of the diet on lipophilic pigments, i.e., lutein and  $\beta$ -carotene, are not available, this study examined these pigments in snails maintained on hen's egg yolk.

### MATERIALS AND METHODS

Twenty juvenile *Biomphalaria glabrata* snails, about 7 mm in shell diameter, were obtained from Dr. Fred Lewis, Schistosomiasis Laboratory, Biomedical Research Institute (Rockville, Maryland, USA). Groups of 10 snails were maintained at 23–24°C in aerated glass containers each containing 800 mL of artificial spring water (ASW) prepared as described by Ulmer (1970). One culture of 10 snails was fed *ad libitum* on boiled Romaine leaf lettuce (L diet). The other culture was fed the hen's egg yolk diet *ad libitum*, supplemented with 500 mg of Romaine lettuce once a week (Y-L diet), as described in Beers et al. (1995). Food and water were changed twice weekly in all cultures.

For TLC analysis, the whole-body of five individual snails ( $n = 5$ ) was prepared for both Y-L and L diets at 14 and 20 days after the cultures were started. To do this, the shell of each snail was gently crushed with a hammer, and the snail body was removed with forceps. Each whole-body was homogenized in 2 mL of acetone in a glass homogenizer. The pellet was washed twice with acetone (100  $\mu$ L), and the washings were combined with the supernatant. The combined supernatant was evaporated to dryness under nitrogen and then reconstituted with 200  $\mu$ L or 300  $\mu$ L of heptane, as necessary for the scan areas of at least one sample zone to be bracketed within the scan areas of the standard zones in the TLC analysis. Single samples ( $n = 1$ ) of the hen's egg yolk (200 mg) and the Romaine lettuce (200 mg) were extracted in acetone and prepared for TLC analysis as described for the snail bodies.

The standards used for TLC analysis were lutein and  $\beta$ -carotene (Sigma, St. Louis, Missouri). The solid standards were weighed on an analytical balance and diluted with dichloromethane to prepare standard solutions of 0.0100  $\mu$ g  $\mu$ L<sup>-1</sup> for both lutein and  $\beta$ -carotene. TLC analyses were performed on Merck (EM Science, Gibbstown, New Jersey) 10 cm  $\times$  20 cm chemically bonded C-18 silica gel plates with concentrating zone (RP-18F<sub>254S</sub>, Art. 15498). Plates were prewashed by development to the top with dichloromethane-methanol (1:1) and dried in air in a fumehood. The standards (4.00, 8.00, 12.0, and 16.0  $\mu$ L for each standard) and 1.00–8.00  $\mu$ L of the reconstituted samples were applied in separate lanes in the concentrating zone by means of a 10- $\mu$ L Drummond (Broomall, Pennsylvania) digital microdispenser in a dark room with minimum lighting. The applied solutions were dried in

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Table 1

Percent weight of lutein and  $\beta$ -carotene in the snails maintained on the yolk-lettuce (Y-L) diet or lettuce (L) diet.

Pigment	Days after the cultures were started	Y-L*	L*
Lutein	14	0.0213 $\pm$ 0.0037 <sup>†</sup>	0.0624 $\pm$ 0.015
	20	0.00833 $\pm$ 0.0039 <sup>a,†</sup>	0.0198 $\pm$ 0.0045 <sup>b,‡</sup>
$\beta$ -carotene	14	0.0410 $\pm$ 0.015 <sup>†</sup>	0.0600 $\pm$ 0.026
	20	0.00840 $\pm$ 0.0030 <sup>c, **</sup>	0.0530 $\pm$ 0.0064 <sup>†</sup>

\* Snail bodies: mean (weight %)  $\pm$  standard error; n = 5 individual snails for each sample (except where indicated).\*\* Concentration significantly reduced (Student's t-test,  $P < 0.05$ ) compared with snails on the L diet.<sup>a</sup> Two data points were below the limit of quantification. Weight % values of 0.00340 and 0.00575 were used for statistical analysis.<sup>b</sup> One data point was below the limit of quantification. Weight % value of 0.00832 was used for statistical analysis.<sup>c</sup> Three data points were below the limit of quantification. Weight % values of 0.00288, 0.00311, and 0.00510 were used for statistical analysis.<sup>†</sup> n = 3.<sup>‡</sup> n = 4.

air for about 30 sec. The plates were developed to a distance of about 7 cm past the concentrating zone-bonded silica gel interface with petroleum ether-acetonitrile-methanol (10 + 20 + 20 v/v) in a rectangular Camag (Wilmington, North Carolina) TLC twin-trough chamber. The chamber was covered with aluminum foil, lined with a saturation pad (Analtech, Newark, Delaware), and equilibrated with the mobile phase for at least 15 min before inserting the plate. Approximately 40 mL of mobile phase was required for each development. The required development time was about 20 min. The plates were briefly dried in air for about 2 min after development. The pigments were detected in visible light as colored bands on a white background.

Quantitative densitometric analysis was performed with a Camag TLC Scanner II with the tungsten light source (set at 448 nm for lutein and 455 nm for  $\beta$ -carotene), slit width 4, slit length 4, and scanning rate 4 mm s<sup>-1</sup>. The CATS-3 software was used to generate a linear regression calibration curve relating the weights of the standard zones (0.0400–0.160  $\mu$ g) to their peak areas. The analyte weight in the sample aliquot with a scan area closest to that of the average of the middle two standard zones was determined by automatic interpolation from the calibration curve, on the basis of its peak area. The weight percents of pigments in the snail whole-body were calculated using the equation described earlier (Sherma et al., 1992).

For quantification of some samples, dilution or concentration were required to obtain scan areas that would be bracketed within the calibration curve. An appropriate correction factor was then included in the calculation. On six occasions after the maximum possible degree of concentration, the largest spotted sample yielded a zone whose area was less than the scan area of the lowest standard zone. Therefore, the exact quantities of the pigment in these zones could not be determined because they

were below the experimental quantification limit, which ranged from 0.00575–0.0166 weight percent of the pigments for the conditions under which the analyses were performed. The reconstitution volume was 200  $\mu$ L for all six samples, with 4.00, 8.00, 4.00, 8.00, 8.00, and 8.00  $\mu$ L aliquot spotted, respectively. For these zones, a concentration of one-half of the limit of quantification was included in the data for statistical calculations (Cline et al., 1999) (see Table 1).

## RESULTS AND DISCUSSION

By comparison with the migration of standards, lutein and  $\beta$ -carotene were identified in chromatograms of the whole snail body extracts from snails fed both the Y-L and L diets at  $R_f$  values of 0.45 and 0.070, respectively. The sample also contained several other pigment zones with different  $R_f$  values, one of which was qualitatively determined to be chlorophyll A.

Table 1 lists quantitative data for lutein and  $\beta$ -carotene in the snails fed both the Y-L and L diet for 14 and 20 days (n = 5 for each sample). The mean values of lutein in the snails on the L diet were  $\times 3$  and  $\times 2.5$  at 14 and 20 days, respectively, compared to snails on the Y-L diet. Likewise, the mean values of  $\beta$ -carotene in snails on the L diet were  $\times 1.5$  and  $\times 6.6$  at 14 and 20 days, respectively, compared to the snails on the Y-L diet. However, the only results that were significantly different (Student's t-test,  $P < 0.05$ ) were those for  $\beta$ -carotene values in the snail tissues 20 days after the cultures were started. By this time, snails on the Y-L diet had significantly reduced amounts of  $\beta$ -carotene than snails on the L diet. The weight percents of lutein (n = 1) from the hen's egg yolk and Romaine leaf lettuce were 0.0730 and 0.253, respectively. The weight percents of  $\beta$ -carotene (n = 1) from the yolk and lettuce were 0.0140 and 0.0830, respectively. The concentration of lutein in the lettuce was approx-

imately  $\times 3.5$  that in the egg yolk. The concentration of  $\beta$ -carotene in the lettuce was approximately  $\times 8$  that in the egg yolk.

The amount of lutein and  $\beta$ -carotene pigments in general reflected the relative amounts of these pigments in the diets. Thus, concentrations of both lutein and  $\beta$ -carotene were higher in the lettuce than the yolk diet, and these higher values were reflected in snails on the L versus Y-L diet. These results are in general accord with a previous study by Eidam et al. (2001) that compared various analytes in the tissue and hemolymph of *Biomphalaria glabrata* fed a diet of Romaine lettuce leaf versus the midrib of the Romaine lettuce. The leafy portion of the Romaine lettuce contained significantly greater amounts of neutral lipids, phospholipids, lipophilic pigments, and carbohydrates than did the midrib portion of the Romaine lettuce. Higher values in these analytes were seen in the tissues and hemolymph of the *B. glabrata* snails fed the leafy portion of the Romaine lettuce. The adage "you are what you eat" is applicable to the *B. glabrata* snails.

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## NOTES, INFORMATION & NEWS

### *Kalidos griffithshauchleri*, sp. nov., Madagascar's Largest Helicarionid Snail (Pulmonata)

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#### Introduction

Owen Griffiths of Mauritius (along with his associates and assistants) was a major participant in the author's 1992–1996 survey and inventory of Madagascar's land mollusks. Griffiths' unique and strongest contribution was in surveying the Reserve Naturelle Integrale de Tsingy de Bemaraha, a little-explored limestone karst region in west-central Madagascar. After some preliminary scouting in 1992 and 1993, Griffiths led expeditions in 1995 and 1996 into the southern and central-plus-northern parts of Bemaraha, respectively (Griffiths, 1995, 1996). Among the many new species of land snails resulting from Griffiths' Bemaraha collections (in Emberton, 1999a, b, 2001, 2002, in press) is the remarkable new *Kalidos* described herein.

The genus *Kalidos* Gude, 1911, is endemic to Madagascar; its sister group has been predicted from biogeographic considerations to lie among the ariophantines of India (Emberton & Rakotomalala, 1996). The *Faune de Madagascar* (Fischer-Piette et al., 1994) listed 71 *Kalidos* species (23 new), Emberton (1994) added one new species, and Emberton & Pearce (2000) added four new species. Thus this current new species brings the total to 77.

The author's 1992–1996 survey and inventory of Madagascar yielded over 2000 lots of *Kalidos* species. Only 438 of these lots have been identified so far, and the 1995–1996 Bemaraha *Kalidos* materials have not been reached yet in this process. However, three specimens of *K. griffithshauchleri*, sp. nov. that were collected in 1992–1993 were sent to the author's attention some time ago and merit description now—in advance of the author's plan to monograph the genus—because of this species' unique size and its conservation implications for Bemaraha Reserve.

The author's identifications of 438 of the some 2000 lots of *Kalidos* have yielded 65 presumed species, of which 42 seem new and undescribed (Emberton, unpublished). Thus Madagascar's total *Kalidos* species now in collections is likely to be at least 250 (contradicting Emberton & Rakotomalala's 1996: table II estimate of "75?"). Most of those species are small, and none begins to approach this new species in its gigantic shell size. All other known and collected Madagascan helicarionids, with the exception of this gigantic Bemaraha species, are much smaller in size

(Fischer-Piette et al., 1994; Emberton 1994; Emberton & Pearce, 2000; Emberton, unpublished).

#### Systematics

Higher classification follows Ponder & Lindberg (1997), Nordsieck (1986), and Vaught (1989). Type materials are placed in the Florida Museum of Natural History, University of Florida, Gainesville (UF) and the Australian Museum, Sydney (AMS). Description follows the format applied to other *Kalidos* by Emberton & Pearce (2000).

#### Class GASTROPODA

#### Clade HETEROBRANCHIA

#### Clade PULMONATA

#### Order STYLOMMATOPHORA

#### Suborder SIGMURETHRA

#### Infraorder HELICIDA

#### Superfamily HELICARIONOIDEA

#### Family HELICARIONIDAE

#### Subfamily ARIOPHANTINAE

#### Genus *Kalidos* Gude, 1911

#### *Kalidos griffithshauchleri* Emberton, sp. nov.

(Figure 1)

*Kalidos* sp. 1, Griffiths, 1995; Griffiths, 1996.

**Diagnosis:** Unique within the genus for its large initial whorls and very rapid whorl-expansion rate producing a gigantic adult shell. *Kalidos griffithshauchleri*, sp. nov. is most similar to *K. bathensis* (Robson, 1914), from which it differs in both its larger initial whorls (diameters of first and first-plus-second whorls = 2.2 mm and 5.1 mm versus 1.7 mm and 3.8 mm) and its looser coiling (whorls/ $\ln$ [diameter] 1.51–1.60 versus 1.76).

**Holotype:** UF285447 (1 adult), Owen Griffiths lot A1680: Madagascar: near Tsingy de Bemaraha: 15 km east of Antsalova: in cave mouth, April 1992.

**Paratypes:** UF285448 (1 adult), type lot. AMS C. 204776 (1 adult), Owen Griffiths lot A1737: Madagascar: near Tsingy de Bemaraha: southeast of Antsalova: near Tsiandro: in cave mouth, April 1993.

#### Description of holotype:

*Shell Size and Shape.* Shell rather thick and robust for

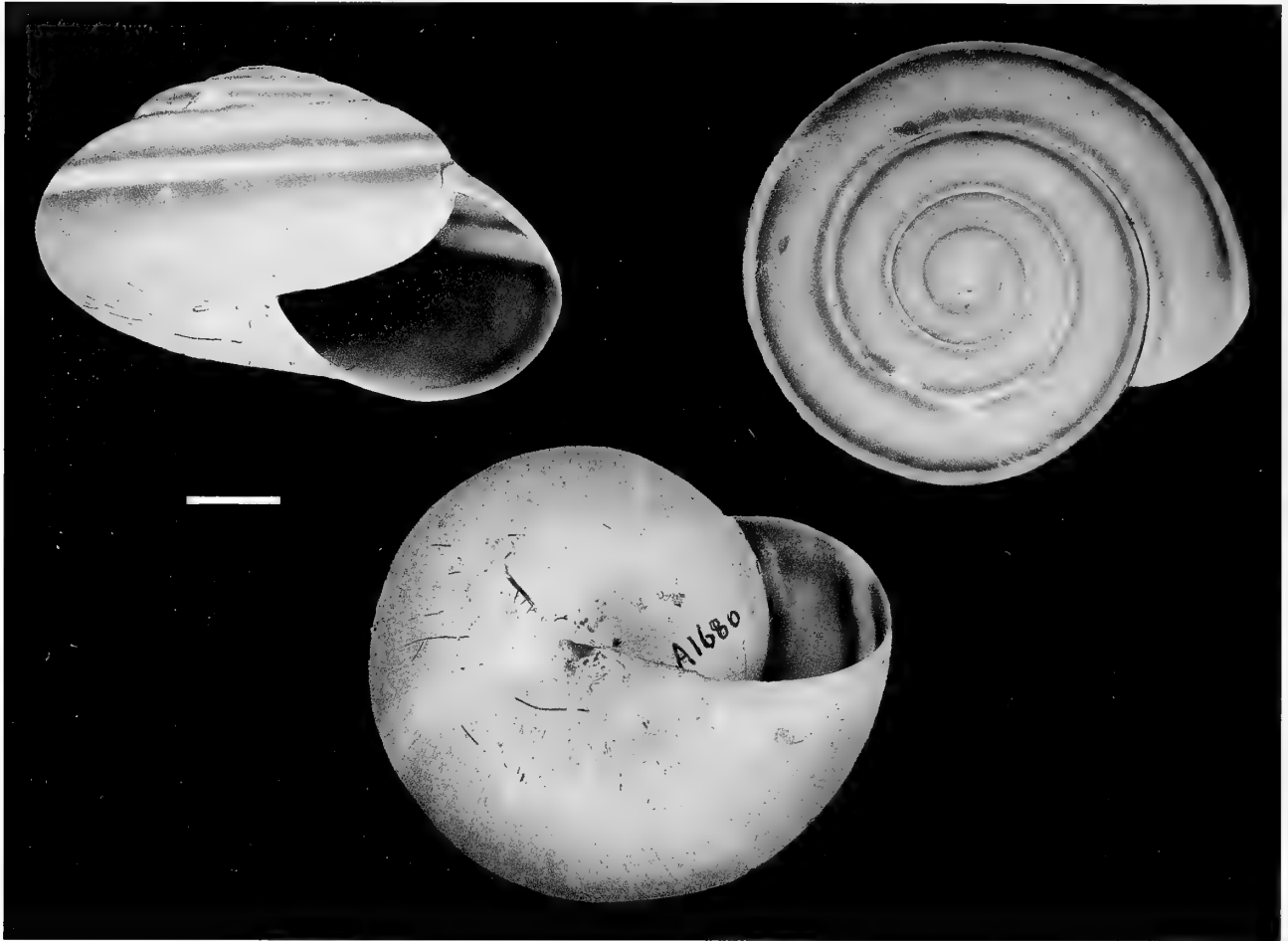


Figure. 1. *Kalidos griffithshauchleri* Emberton, sp. nov., holotype. Scale bar = 10 mm.

the genus. Diameter 58.5 mm, height 38.4 mm (h/d 0.66). Whorls 6.5 (coiling tightness = whorls/ $\ln$ (diameter) = 1.6). Spire angle 155 degrees. Shell domed. Whorl periphery rounded. A faint, rather narrow, subsutural, spiral gutter is present throughout ontogeny. Suture depth one-half whorl from aperture is 1.4% of shell diameter. Subsutural line (where inside of shell wall meets previous whorl) not visible through shell. Umbilicus 3% of shell diameter, half covered by columellar reflection of apertural lip. Shell color whitish above, and a light yellowish brown below that grades to whitish on the base, marked both by a very conspicuous supraperipheral spiral band that is white, sharply bordered above and below by dark brown to purple-brown, and by a narrower and less conspicuous subsutural spiral band that is white bordered below by dark brown to purple-brown.

**Aperture.** Aperture width (measured parallel to a line between the columellar and upper peristome insertions) 45% of shell diameter. Aperture height-width ratio (height measured to and perpendicular to a line between the columellar and upper peristome insertions) 0.90. Distance

between the columellar and upper peristome insertions 87% of aperture width. Penultimate whorl projects into body whorl, occupying 23% of aperture height. Lower peristome angle where it meets parietal wall (apertural view) 20 degrees.

**Apex.** First whorl diameter 2.2 mm. First two whorls diameter 5.1 mm. Embryonic whorls 2.1. Embryonic sculpture (partially eroded) of close-set, dense, wrinkled axial striae crossed by dense, fine spiral grooves.

**Post-Embryonic Shell Sculpture.** Close-set, obliquely axial striae, somewhat uneven in width, crossed by close-set spiral grooves to produce a pustulose appearance. Spiral grooves and their resulting pustules fading below the shell periphery, absent from the base, where only axial striae are visible.

#### Variation:

	Diameter	Ht/Diam	Whorls	Wh/ $\ln$ Diam
Holotype	58.5	0.66	6.5	1.60
"Paratopotype"	56.3	0.60	6.1	1.51
Paratype	57.0	0.62	6.2	1.53

Griffiths (1996) reported a maximum diameter of 65 mm in the central and northern parts of Bemaraha Reserve.

The "paratopotype" is the freshest shell, with embryonic sculpture much more sharply detailed than in the holotype or other paratype.

**Distribution:** Bemaraha Reserve and its karstic vicinity, from the Manombolo River north to at least opposite the town of Antsalova, latitudes 18°02'–19°08'S, longitudes 44°32'–44°53'E (Griffiths, 1995, 1996).

**Ecology:** Griffiths (1995, 1996) reported, "This is the most obvious tsingy [=limestone karst] snail at Bemaraha. It can be found dead all over the tsingy in large numbers. Aestivates deep inside narrow tsingy slots where it sticks itself firmly to the substrate."

**Etymology:** For this species' co-discoverers, Owen Griffiths and Jorg Hauchler, both of Mauritius.

**Acknowledgments.** The staff at Ranomafana National Park Project in Antananarivo helped in getting collecting and export permits. Owen Griffiths' field surveys of Bemaraha were aided especially by Jorg Hauchler, Vincent Florens, and Roger Randallana.

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#### Fungi and Other Items Consumed by the Blue-Gray Taildropper Slug (*Prophysaon coeruleum*) and the Papillose Taildropper Slug (*Prophysaon dubium*)

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#### Introduction

Six species of slugs, in addition to 29 other aquatic and terrestrial mollusk species, were listed in the Record of Decision for the Northwest Forest Plan (USDA and USDI, 1994). They were included in a list of rare taxa associated with late successional forests, referred to as Survey and Manage species, that require additional mitigation in order to assure their persistence. These species were listed, in part, due to the lack of information on their natural history and ecology.

Two Survey and Manage slug species were the focus of this study: the blue-gray taildropper (*Prophysaon coeruleum* Cockerell, 1890) and the papillose taildropper (*P. dubium* Cockerell, 1890). Studies have shown slugs of other species to be mycophagists (Buller, 1922; Chatfield, 1976; Pallant, 1969). Field observations of these two *Prophysaon* slug species on and within partially eaten fungi suggested that they are also mycophagous. We tested this hypothesis by examining fecal pellets from these slug species for evidence of ingested fungal material.

#### Materials and Methods

*P. coeruleum* and *P. dubium* were collected during field surveys within several proposed timber sale areas in Douglas County, Oregon on Bureau of Land Management lands from March 1998 through May 1999. These were

Table 1

Frequency of food item occurrence in fecal samples of *Prophysaon coeruleum* and *Prophysaon dubium*.  
(Total # of samples and % of samples containing item).

Single food item	<i>Prophysaon coeruleum</i>						<i>Prophysaon dubium</i>					
	Spring (n = 34)		Fall (n = 52)		Spring and fall (n = 86)		Spring (n = 20)		Fall (n = 37)		Spring and fall (n = 57)	
Plant tissue	25	74%	26	50%	51	59%	14	74%	26	59%	40	63%
Lichens	0	0	13	25%	13	15%	2	10%	8	18%	10	16%
Imperfect fungi	4	12%	6	12%	10	12%	5	26%	4	9%	9	14%
Fungal hyphae*	25	74%	42	81%	67	78%	11	58%	32	73%	43	68%
Fungal spores*	8	24%	32	62%	40	47%	1	5%	37	84%	38	60%
Unidentified	0	0	2	4%	2	2%	2	10%	1	8%	3	5%

\* Data does not include imperfect fungi.

predominantly Douglas fir timber stands ranging in age from 50 years to over 200 years old with average tree diameters at breast height (DBH) of 50 cm to over 100 cm. The majority of fecal samples were collected from slugs located in stands over 80 years of age. Surveys were done during the spring and fall when the forest litter layer was moist and the ambient air temperature was between 4°C and 11°C. The established protocol for Survey and Manage terrestrial mollusks (Furnish et al., 1997) was followed. Time-constrained surveys were conducted in suitable habitat with emphasis on suspected areas of high-quality habitat. Two 81 m<sup>2</sup> plots in every 4 hectares of project area, specifically located in high-quality habitat, were intensively searched for 20 minutes each. Another 20 minutes was spent at other sites throughout the remainder of the 4 ha conducting brief, 1–5 minute opportunistic searches.

Specimens of either *P. coeruleum* and *P. dubium* were placed individually in clean, white film canisters until they produced fecal pellets (typically within 1–4 hours). Fecal pellets from individual animals were taken as they were produced from the animal or were collected where they fell on the surfaces of the canisters. No substrate or plant material from the discovery site was placed in the canister with the animal. Fecal pellets were removed from the canisters and immediately placed in a vial of 70% isopropyl alcohol. The animals were returned to the site of collection or kept as vouchers. Identification of slug species was done by examination of external physical characteristics only. Only specimens which conformed to the described species were used in this study. Voucher specimens currently reside at the Roseburg, Oregon Field Office of the Bureau of Land Management.

For fecal analysis, pellets were moved to small vials of 50% ethanol to dissolve lipid layers of viruses which might pose health threats to humans (Colgan et al., 1997). One to two drops of distilled water were then added to rehydrate the samples for 48 hours at room temperature. Pellets were macerated and mixed thoroughly. The resulting suspension was transferred to a microscope slide.

One to two drops of Melzer's reagent (iodine, potassium iodide, and chloral hydrate in aqueous solution) were added and the suspension then covered with a 22 × 22 mm cover slip. One slide was made per sample. Seventy-five fields, each 450 µm in diameter, across three horizontal lines of view were then examined on each slide at 250× magnification with a compound microscope. Fungal spores were identified to family, genus, or species according to Castellano et al. (1989). Plant material, lichens, molds, fungal hyphae, and other fungal structures, as well as occasional arthropod fragments and nematodes, were recorded.

Quantitative analysis of the frequency of detections of ingested material was not the intended focus of this study, and the methods used were not quantitatively rigorous. For instance, fecal pellets were not equal in volume, resulting in unequal dilutions in slide preparations. However, an apparent difference was observed in the proportions of fungal and plant material detected in spring samples as compared to fall samples. We investigated this trend using Chi-square tests to detect significant differences ( $\alpha = 0.05$ ) in the frequency of the types of materials identified between fall and spring seasons, and between slug species within seasons. No significance tests were done on the fungal taxa due to the small sample sizes within several fungal taxa.

## Results and Discussion

Both *Prophysaon* species in this study showed evidence of consumption of fungi (spores or hyphae of mushrooms or truffles), vascular plant material (both root tissue and other plant tissue), lichens, and imperfect fungi, i.e., molds in their fecal samples (Table 1). Fungi were the most common items found in both *P. coeruleum* 90% (77/86) and *P. dubium* 82% (47/57) samples, with spores from 10 separate fungal families identified. In addition, fragments of arthropods were found in 8% (11/143) of the samples, and nematodes were found in 6% (8/143) of the samples. Nematodes were seen to be whole and in



Table 2

Summary of fungal spore frequency in fecal samples of *Prophysaon coeruleum* and *Prophysaon dubium*.

Fungal spore identity	<i>Prophysaon coeruleum</i>				<i>Prophysaon dubium</i>			
	Spring (n = 35)		Fall (n = 59)		Spring (n = 19)		Fall (n = 63)	
Subclass: Ascomycotina*	1	3%	5	8%	1	5%	4	6%
order: Tuberales	1	3%	5	8%	1	5%	4	6%
family: Tuberaceae	1	3%	5	8%	1	5%	4	6%
genus: <i>Genea</i>	0	0	4	7%	0	0	3	5%
<i>Hydnотrya</i>	0	0	0	0	1	5%	0	0
<i>Pachyphloeus</i>	0	0	1	2%	0	0	0	0
<i>Tuber</i>	1	3%	0	0	0	0	1	2%
Subclass: Basidiomycotina	6	17%	27	46%	0	0	30	48%
order: Ramariales	0	0	1	2%	0	0	1	2%
family: Ramariaceae	0	0	1	2%	0	0	1	2%
genus: <i>Gautieria</i>	0	0	1	2%	0	0	1	2%
order: Agaricales	6	17%	26	44%	0	0	29	46%
family: Bolbitiaceae	2	6%	5	8%	0	0	7	11%
family: Boletaceae	2	6%	5	8%	0	0	7	11%
genus: <i>Melanogaster</i>	2	6%	3	5%	0	0	5	8%
family: Rhizopogonaceae	2	6%	6	10%	0	0	3	5%
genus: <i>Rhizopogon</i>	2	6%	6	10%	0	0	3	5%
family: Coprinaceae	0	0	1	2%	0	0	1	2%
family: Cortinariaceae	0	0	5	8%	0	0	5	8%
genus: <i>Hymenogaster</i>	0	0	0	0	0	0	1	2%
family: Entolomataceae	1	3%	0	0	0	0	0	0
family: Russulaceae	1	3%	4	7%	0	0	6	10%
genus: <i>Gymnomyces</i>	1	3%	4	7%	0	0	6	10%
Subclass: Zygomycotina	1	3%	2	3%	0	0	0	0
order: Glomales	1	3%	2	3%	0	0	0	0
family: Glomaceae	1	3%	2	3%	0	0	0	0
genus: <i>Glomus</i>	1	3%	1	2%	0	0	0	0
genus: <i>Sclerocystis</i>	0	0	1	2%	0	0	0	0

\* Numbers given for a subclass, order, or family include both specimens identified to genus as well as those identified only to their respective family or order.

good condition, suggesting that they were internal parasites rather than food items. There was no evidence indicating that *P. coeruleum* and *P. dubium* had different diets at this level of resolution.

While acknowledging that the methods used were not quantitatively rigorous, the data suggest a shift in the diet of both species between spring and fall (Table 1). Both species appear to ingest plant material more frequently in spring than in fall. Fungal hyphae, spores, and lichens were more frequently consumed in fall than spring. Chi-square analysis indicates that *P. coeruleum* had significantly more plant material in its fecal samples in the spring than in the fall ( $\chi^2 = 4.716$ ,  $df = 1$ ,  $P = 0.030$ ), but had more lichens ( $\chi^2 = 10.014$ ,  $df = 1$ ,  $P = 0.002$ ) and fungal spores ( $\chi^2 = 11.938$ ,  $df = 1$ ,  $P = 0.001$ ) in the fall than in the spring. *P. dubium* samples had significantly more spores in the fall than in the spring ( $\chi^2 = 22.185$ ,  $df = 1$ ,  $P < 0.001$ ).

Spores from taxa in the order Agaricales were most commonly recorded. Most of the fungal spores identified (Table 2) were from mycorrhizal taxa that are root sym-

bionts with vascular plants (including many conifer species) and whose hyphae are attached to the rootlets of such plants. In addition, most of the samples with fungal spores identified were of hypogeous fungal species (49/78). The term hypogeous, as used here, includes those species with fruiting bodies occurring within the forest duff layer as well as in mineral soil, such as truffles. All of the other epigeous spore species identified are in the order Agaricales. Twenty-five of the 29 samples containing epigeous fungal spores were collected in the fall, which may help to account for the increased proportion of fungal material in fall samples.

Fungal and vascular plant material appear both separately and together in individual fecal samples. We identified plant tissue composed of root cells and also green plant tissue containing chloroplasts and amyloid granules. Green plant matter was present in the absence of fungal material in 10% (14/143) of the samples, but root tissue was never observed in samples that did not contain fungal hyphae. Fungal material was observed in the absence of plant matter in 37% (53/143). Both plant tissue and fun-

gal tissue were found together in 52% (75/143) of the samples.

These slug species are commonly observed in the forest floor litter layer or associated with coarse woody debris into which conifer roots commonly penetrate. We hypothesize that due to the intimate connections of mycorrhizal hyphae with plant rootlets, root material may have been ingested during the process of foraging for these fungal hyphae. Green plant matter may have been ingested either due to its intrinsic food value or due to the presence of bacteria or yeasts on the surfaces of decomposing material. The presence of spores in 55% (78/143) of the fecal samples suggests that fungal fruiting bodies were being deliberately targeted because these structures are not typically closely associated with plant roots. Fecal pellets collected from four other mollusk species, i.e., *Ariolimax columbianus* (Gould, 1851), *Prophysaon andersoni* (Cooper, 1872), *Prophysaon vannatta* (Pilsbry, 1948), and *Megomphix hemphilli* (Binney, 1879) also evidenced ingestion of both plant and fungal material.

The relative importance of plant, fungal, and other material in the diets of these two slug species warrants further investigation; however, *P. coeruleum* and *P. dubium* in this region are clearly at least partially mycophagous, and especially so in the fall. Most of the fungal species identified are mycorrhizal and hypogeous. Fungal fruiting bodies seemed to be targeted as food items, however fungal hyphae were also present in most samples. Spores seemed to be in good condition, and these slug species may be important vectors for spore dispersal of these forest fungi (Kimmerer & Young, 1995). Future viability studies on the hyphal fragments in mollusk fecal pellets may indicate that dispersal of live hyphae may also be occurring.

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#### The Taxonomic Status of the Freshwater Snail *Antillobia margalefi* Altaba, 1993, from Hispaniola (Hydrobiidae: Cochliopinae)

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A recent paper (Altaba, 1993) described a freshwater snail from Lago de Enriquillo, Dominican Republic, as *Antillobia margalefi*, new genus and new species. The description is based on "very few specimens," of which two males and two females were dissected. The specimens were preserved unrelaxed in the field in 10% formalin and later transferred to 70% ethanol. They were highly contracted and distorted within their shells because of how they were preserved. The holotype and two figured paratypes were deposited in the Museu de la Naturalesa de les Illes Balears, Ciutat de Mallorca. A third figured paratype remained in the author's collection. Other uncited and unfigured paratypes were said to be in the Florida Museum of Natural History, but they cannot be located.

Altaba (1993) used 27 character-states to compare *Antillobia* with three closely related genera, *Spurwinkia* Davis, Mazurkiewicz & Mandracchia, 1982, *Heleobia* Stimpson, 1865, and *Heleobops* Thompson, 1968 (Cochliopinae) and with the distantly related genus *Hydrobia* Hartmann, 1821 (Hydrobinae). Anatomical data for the four genera were taken from the literature, and were based on abundant specimens that had been properly relaxed and fixed prior to preservation. The 27 character-states are as follows.

1. Hypertrophied ciliation of left tentacle simple (0), grouped in transversal bands (1), or forming subdivided transversal bands (2).
2. Mantle edge with (0) or without (1) pallial tentacle.

3. Osphradium annular (0) or voluted (1).
4. Posterior caecum of stomach deep and bent laterally (0), or median and shallow (1), or altogether absent (2).
5. Typhosole **d** and the dorsal groove it defines absent (0) or present (1).
6. Opening of anterior digestive gland absent (0), anterior (1), posterior (2), or fused with that of posterior digestive gland (3).
7. Gastric shield small (0) or large (1).
8. Ovary lobes few and wide (0), or few globose (1), or several wide (2), or numerous digitiform (3).
9. Anterior end of ovary covering stomach (0) or posterior to it (1).
10. Posterior end of ovary reaching close to posterior end of body (0), or placed far from it (1).
11. Ovary entering ventral sperm canal (0) or posterior pallial oviduct (1).
12. Oviduct coiled (0), or just bent over itself (1).
13. Pallial oviduct divided into two (0) or three (1) distinct regions.
14. Albumen gland straight (0) or bent on itself (1).
15. Lobes of albumen gland small (0), or large and columnar (1).
16. Spermathecal duct absent (0) or present (1).
17. Spermathecal duct coalesced with (1) or independent of (2) oviduct.
18. Spermathecal duct long (1) or short (2).
19. Duct of seminal receptacle stemming off of oviduct (0), connected to it by a short sperm duct (1), or through a simple orifice where they are oppressed (3).
20. Uneverted penis straight (0) or coiled (1).
21. Terminal papilla on the verge simple (0) or eversible (1).
22. Surface of verge smooth (0), or creased and glandular (1).
23. Globose glands on convex side of verge absent (0) or present (1).
24. Stalked glands on convex side of verge absent (0), short (1), or cuplike (2).
25. Anterior concave side of verge with a non-glandular lobe (0), or with a lobe carrying discrete glands along its edge (1), or with a lobe of glandular tissue (2), or without such a lobe (3).
26. Subterminal ciliation on the verge present (0) or absent (1).
27. Longitudinal groove on verge absent (0) or present (1).

Six of the character-states used by Altaba are non-variable within the four cochliopine genera and have no comparative value except to separate the Cochliopinae from the Hydrobiinae. These are character-states 2, 5, 7, 11, 13, and 16.

Eight character-states also have little comparative value because of the manner in which the specimens were preserved and how these character-states were interpreted. For example, the head is illustrated as though it were in a natural relaxed condition (Altaba: 1993, fig. 3), yet if the specimens had been killed unrelaxed in formalin, the head and tentacles would have been severely contracted. Surely the head as illustrated is an interpretation and not an actual depiction. Interpretations of eight character-states used to separate *Antillobia* from *Heleobops* are questionable for the same reason. These include: (3) the

shape of the osphradium, (4) the size and shape of the posterior caecum of the stomach, (6) the opening of the digestive gland, (14) the shape of the albumen gland, (22) the texture of the penis surface, and (24) having unstalked or weakly stalked glands along the convex side of the penis. *Antillobia* is described as having discrete glands along the anterior concave side of verge (25). The depiction of these structures in Altaba: 1993, fig. 9 is non-convincing as glands and not as contracted folds of skin. *Antillobia* is said to have a longitudinal groove on [the dorsal surface of] the penis (27). This also is an artifact of preservation due to intense contraction and partial desiccation of the animals caused by having been killed and fixed in formalin.

Five character-states pair *Antillobia* with *Heleobops*, and separate the two from *Spurwinkia* and *Heleobia*. These are: the ciliation pattern on the tentacles (1-1), the anterior extent of the ovary over the stomach (9-1), the weak coiling of the oviduct (12-1), the short spermathecal duct (19-2), and the presence of globose glands along the convex side of the verge (23-1).

Five-character states group *Antillobia* and *Heleobops* with *Heleobia*, and separate the three from *Spurwinkia*. These are: the independent spermathecal duct from the oviduct (17-2), the short spermathecal duct (18-2), the coiled uneverted penis (20-1), the simple terminal papilla on the verge (21-0), and the absence of subterminal ciliation on the verge (26-1).

Three character-states are left to separate *Antillobia* from *Heleobops*. These are (8) the size and number of the ovary lobes, (10) the location of the ovary within the digestive gland, and (15) the size and shape of the albumen gland lobes. These three characters hardly constitute a basis for separating genera, especially considering that the data are based only on two inadequately preserved specimens.

Two taxonomic questions are posed by the description of *Antillobia margalefi*. One question is the status of the genus name *Antillobia*, and the other question is the status of the species name *margalefi*. The description and illustrations given for *Antillobia* pertain to two previously known species, *Pyrgophorus coronatus bermudezi* (Aguayo, 1947) and *Heleobops clytus* Thompson & Hershler, 1991. Both are common species about Lago de Enriquillo, and they are the only two hydrobiids known to occur there. *Heleobops clytus* is oviparous with the uterus unmodified into a brood pouch, and the verge (penis) bears unstalked apocrine glands along its outer curvature. *Pyrgophorus coronatus bermudezi* is viviparous, with a brood-pouch containing developing juveniles, and the verge bears elongate papillae along the outer curvature and elsewhere, as is typical for *Pyrgophorus* (Hershler & Thompson, 1992:90-91). The female reproductive anatomy of *Antillobia*, as described and figured by Altaba, lacks a brood pouch with developing embryos and juveniles (Altaba, 1993, fig. 4), as is typical for *Heleobops*.

The penis, as described by Altaba, has unstalked apocrine glands along its outer curvature. The anatomical data presented by Altaba for *Antillobia* pertains to *Heleobops*.

Under normal circumstances, the anatomical information would resolve the identity of *A. margalefi*, but shell features add confusion. The designated holotype is a "large" intact specimen in alcohol (approx. 2.4 mm long and consisting of five whorls). Shells of *H. clytus* and *P. c. bermudezi* are very similar to each other, and sometimes it is not possible to separate the shells of immature specimens (see Thompson & Hershler, 1991:674). The shell length and number of whorls of the holotype suggest a juvenile male of either species. Both species are sexually dimorphic in size, with females being much larger than males with the same number of whorls. Females 2.4 mm in length of either species typically have four or fewer whorls. The description of the shell seems to be a composite of characters from two species. *Antillobia margalefi* is described as having slightly swollen whorls with a moderately deep suture. *The periostracum is pale ochre in color* and has numerous exceedingly faint, thin, irregularly spaced spiral striations. *The peristome is continuous across the parietal margin*. The traits in italics are typical for *P. c. bermudezi*. The other traits are typical for *H. clytus*. The figure of the holotype of *A. margalefi* (Altaba, 1993, fig. 1) is most similar in appearance to typical male *P. c. bermudezi*. However, the specific identity cannot be resolved unless the holotype is dissected, which undoubtedly would require destroying the shell. Under the circumstances, the name *Antillobia margalefi* must be considered a synonym of *Pyrgophorus coronatus bermudezi* because of the holotype shell, even though the anatomical data given by Altaba pertain to *Heleobops clytus*.

The following taxonomic changes are in order.

(1) The species name *Antillobia margalefi* Altaba, 1993, is a subjective junior synonym of *Pyrgophorus coronatus bermudezi* (= *Lyrodes coronatus bermudezi* Aguayo, 1947).

(2) The genus name *Antillobia* Altaba, 1993, is a subjective junior synonym of *Pyrgophorus* Ancy, 1888, because its type species is a *Pyrgophorus*.

(3) The anatomical data presented by Altaba, which pertain to *Heleobops clytus*, do not justify separation of *Heleobops clytus* Thompson & Hershler, 1991, as a genus distinct from *Heleobops docimus* Thompson, 1968, the type species of *Heleobops*. Synonymies for the two species are as follow.

*Pyrgophorus coronatus bermudezi* (Aguayo, 1947)

*Lyrodes coronatus bermudezi* Aguayo, 1947:81–83; fig. 1 (holotype), fig. 2 (paratypes).

*Pyrgophorus coronatus bermudezi* (Aguayo), Thompson & Hershler, 1991: 679; fig. 4. Hershler & Thompson, 1992:92.

*Antillobia margalefi* Altaba, 1993: 73, 90; fig. 1.

**Type locality:** *Lyrodes coronatus bermudezi*: República Dominicana, Provincia de Barahona, Lago Enriquillo, cerca de Mella (now in Provincia Independencia), Pleistoceno (?). Holotype: Museo Poey, no. 12146. *Antillobia margalefi*: Lago Enriquillo (*sic*), Dominican Republic. Holotype: Museu de la Naturalesa de les Illes Balears, Ciutat de Mallorca, uncatalogued.

**Distribution:** Known only from the vicinity of Lago de Enriquillo. Other forms of *Pyrgophorus coronatus* are found elsewhere on Hispaniola.

**Specimens examined:** DOMINICAN REPUBLIC. *Independencia Prov.*: spring 2 km ESE of Duverge (UF 175165); spring, 6 km WNW of Duverge (UF 174885, preserved UF 252046); spring, Boca de Cachón (UF 175215); spring, 4 km E of La Descubierta (preserved UF 252048); spring, 1 km W of Las Baitoas (UF 174889).

*Heleobops docimus* Thompson & Hershler, 1991

*Heleobops clytus* Thompson & Hershler, 1991: 672–674; fig. 3. Hershler & Thompson, 1992; Malacological Review, Suppl. 5:60; figs. 34, 35a.

*Antillobia margalefi* (in part) Altaba, 1993: 73, 90; figs. 3–10.

**Type locality:** A spring 2 km ESE of Duverge, Independencia Prov., Dominican Republic. Holotype: UF 175170.

**Distribution:** At present known only from the environs of Lago de Enriquillo and the Laguna del Rincón, Dominican Republic. All of these stations are in the *cul de sac* that extends from Barahona, Dominican Republic to Port-au-Prince, Haiti. *Heleobops* is widespread elsewhere on Hispaniola, but the taxonomy of these other populations has not been resolved yet.

**Specimens examined:** HISPANIOLA. DOMINICAN REPUBLIC. *Independencia Prov.*: spring 2 km ESE of Duverge (holotype, paratypes UF 135428, 174880, preserved UF 93973); Laguna Del Rincon, 6 km. WNW Cabral (UF 175203, UF 174884, preserved UF 93976); spring 5 km WNW Duverge (UF 174881, UF 175202); spring, Boca de Cachón (UF 174891, preserved UF 93974); spring 1 km W of Las Baitoas (UF 174887, preserved UF 175199); spring along N shore Lago de Enriquillo, 4 km E La Descubierta (UF 174894, preserved UF 175201); Laguna La Sequia, 1 km S of Augustura (UF 45687); spring, 4 km ENE Neiba (UF 174896, preserved UF 175200).

Lago de Enriquillo is fed by seasonal rivers and by numerous springs along the south, west, and north shores. The two hydrobiid species are abundant on vegetation in springs and spring-fed streams that drain into the lake. The more saline environ of the lake is nearly devoid of aquatic angiosperms, and snails are very sparse or absent there.

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**Predation of Water Bug *Sphaerodema rusticum* on the Freshwater Snails *Lymnaea (Radix) luteola* and *Physa acuta***

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The freshwater snails *Lymnaea (Radix) luteola* Lamarck, 1822, and *Physa acuta* Draparnaud, 1805, are found side by side in nature where they are occurring. It is practically impossible to distinguish them at a glance. The water bug *Sphaerodema rusticum* Fabr. preys upon both the snail species (Raut et al., 1988; Aditya & Raut, in press). Since the prey individuals are of similar type with respect to their shell contour and size, the aim of this study was to determine whether the water bug *Sphaerodema rusticum* has preference for either of the species and, if so, whether the water bug is able to select the individuals belonging to the preferred prey species when both the prey species are found together. The snail *L. (R.) luteola* is involved in the spread of worm diseases in man and animals (Raut, 1986, 1991; Subba Rao, 1989; Mukhopadhyay, 1991; Srivastava, 1991; Subba Rao & Mitra, 1991), and *P. acuta* is causing serious problems in sewage purification plants (Macha, 1971). Attempts are being made to control these snails through the use of biological agents. Therefore, the findings of the present study will enable us to gain some knowledge on the effective use of the water bug *S. rusticum* to control the snails *L. (R.) luteola* and *P. acuta*.

## Materials and Methods

A large number of *L. (R.) luteola* and *P. acuta* 6-7 mm in shell length were collected from the municipality

drains in Kolkata, India. The adult morphs of the water bug *S. rusticum* were also collected from the same drain simultaneously. They were kept in the laboratory in pond water, in plastic containers. The snails were fed with lettuce regularly for a period of 7 days. The water bugs were allowed to feed on the snails kept in the containers. After 1 week, the following experiments were performed to note the rate of predation of *S. rusticum* on the prey individuals supplied.

- Experiment I. 40 *L. (R.) luteola* were exposed to an adult *S. rusticum*.
- Experiment II. 40 *P. acuta* were exposed to an adult *S. rusticum*.
- Experiment III. 40 prey individuals (20 *L. (R.) luteola* and 20 *P. acuta*) were exposed to an adult *S. rusticum*.

The same-sized *L. (R.) luteola* and *P. acuta* were almost equal in weight.

Experiments were carried out in plastic containers, each 25 cm in diameter and 8 cm in depth, containing 2.5 L pond water. All the experiments were carried out for 7 consecutive days. Experiments with the single prey species were repeated three times, while those with the combination of two prey species were repeated six times. Data were collected on the number of snails consumed completely (except the shell) and partially, at the end of each 24 hour period. The water in the container was replaced by fresh pond water, and the prey snail individuals, as per specification were released into the container every 24 hours. In all cases, mean and standard error ( $\pm$  SE) were calculated. Analysis of variance (ANOVA) was applied (Campbell, 1989) to ascertain whether the rate of predation differed significantly with the prey species, singly, or in combinations of the two, or not.

## Results

## Experiment I

In 21 trials the adult *S. rusticum* killed a total of 262 *L. (R.) luteola*. Of these, 179 (68.32%) and 83 (31.68%) were devoured completely and partially, respectively, by the water bug. The water bug killed 8-18 (average  $12.48 \pm 0.65$ ) individuals per day. The number of completely and partially consumed individuals ranged from 6-10 (average  $8.52 \pm 0.31$ ) and 0-9 (average  $3.96 \pm 0.51$ ) per day (Figure 1), respectively.

## Experiment II

The water bug killed a total of 217 *P. acuta* in 21 trials in 7 days. The number of completely and partially devoured individuals was 82 (37.79%) and 135 (62.21%), respectively. The daily rate of predation ranged from 7-16 (average  $10.33 \pm 0.56$ ). Of these, 0-10 (average  $3.9 \pm 0.45$ ) and 2-14 (average  $6.43 \pm 0.7$ ) individuals were

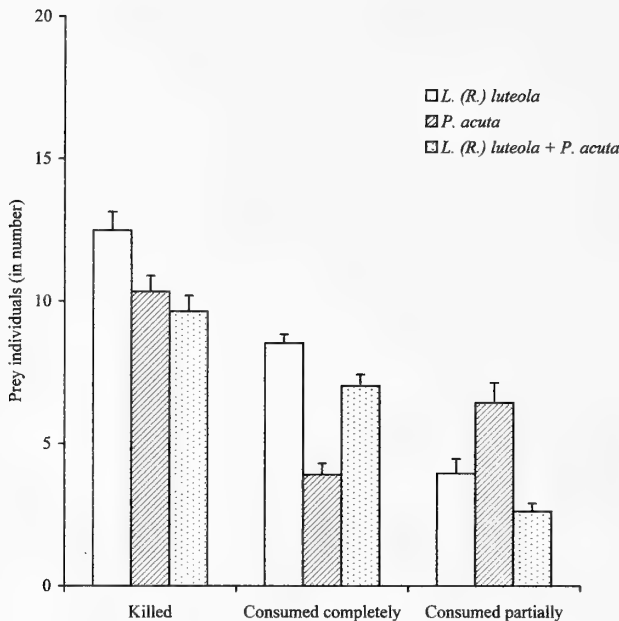


Figure 1. The number (mean  $\pm$  SE) of prey individuals belonging to *L. (R.) luteola* and *P. acuta* killed, completely consumed, and partially consumed per day (24 hours) by an adult *S. rusticum* (40 individuals of each prey species were supplied separately for 24 hours).

devoured completely and partially, respectively (Figure 1).

### Experiment III

Irrespective of prey species, a total of 405 individuals were killed by the water bug in 42 trials. Of these, 295 (72.84%) and 110 (27.16%) individuals were devoured completely and partially, respectively. The daily rate of predation, irrespective of prey species, ranged from 4–19 (average  $9.64 \pm 0.55$ ), and the number of completely and partially consumed individuals ranged from 4–14 (average  $7.02 \pm 0.4$ ) and 0–11 (average  $2.62 \pm 0.28$ ) per day, respectively (Figure 1).

Analysis of the data revealed that the water bug killed 263 *L. (R.) luteola* and 142 *P. acuta* in 42 trials. Of the 263 *L. (R.) luteola*, 229 (87.07%) and 34 (12.93%) were consumed completely and partially, respectively. The water bug consumed 66 (46.48%) and 76 (53.52%) *P. acuta* completely and partially, respectively. A comparative account of the rate of kill and consumption, completely and/or partially by the water bug is shown in Figure 2.

ANOVA tests clearly revealed no significant difference in the rate of predation in terms of killing of the prey individuals per day by *S. rusticum* between the prey snail species *L. (R.) luteola* and *P. acuta*. However, the rate of complete consumption of the prey individuals by the predator differs significantly ( $P < 0.01$ ) with the prey species. Similarly, the difference in partially fed individ-

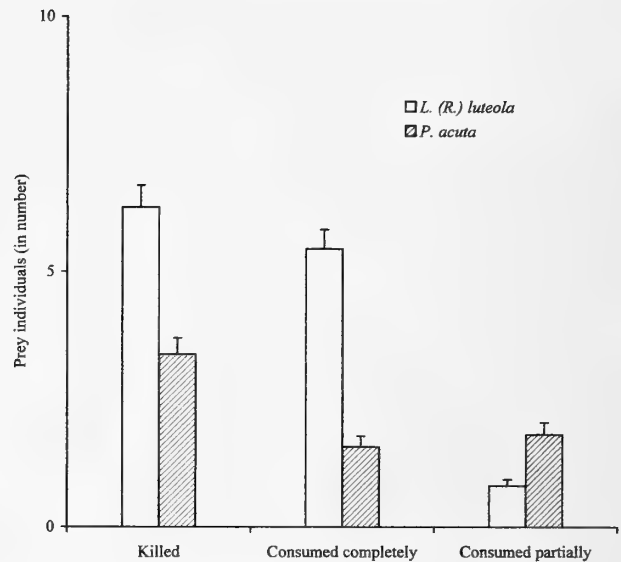


Figure 2. The number (mean  $\pm$  SE) of *L. (R.) luteola* and *P. acuta* killed, completely consumed, and partially consumed by an adult *S. rusticum* per day (24 hours) when 20 *L. (R.) luteola* and 20 *P. acuta* were supplied together.

uals between the prey species is statistically significant ( $P < 0.01$ ). In *L. (R.) luteola* the difference in the number of completely and partially consumed individuals is statistically significant ( $P < 0.01$ ). In the case of *P. acuta*, however, such differences are insignificant. The water bug, while exposed to both the prey species, killed a varying number of individuals with respect to species. Such variations are statistically significant ( $P < 0.01$ ) as is evident from the results of ANOVA tests. Also, the variations in the rate of completely consumed ( $P < 0.001$ ) and partially consumed ( $P < 0.05$ ) prey individuals are statistically significant with respect to the prey species concerned.

### Discussion

The water bug *S. rusticum* killed on an average 12.48 and 10.33 *L. (R.) luteola* and *P. acuta*, respectively, when they were supplied separately in equal numbers daily. Although the rate of killing of the prey snails varied with the treatment, such variations are statistically insignificant. Therefore, it appears that both species of prey snail were almost equally acceptable to the water bug *S. rusticum*. However, it appears that *S. rusticum* is sensitive to the quality of the food materials of the snail species concerned. It consumed 68.27% and 37.75% of the captured (killed) *L. (R.) luteola* and *P. acuta* completely, respectively, daily when predation was confined to the individuals belonging to a single prey species. It is difficult to accept the idea that the quantity of food present in an individual *P. acuta* is double the amount contained in a same-sized (equal weight) *L. (R.) luteola*. If that were the

case, why did the predator feed on 62.25% *P. acuta* partially? In the case of predation on both prey species, *S. rusticum* killed 6.26 *L. (R.) luteola* and 3.38 *P. acuta* per day. Since there were 40 prey individuals, 20 *L. (R.) luteola* and 20 *P. acuta*, the water bug would have consumed only *L. (R.) luteola* to satisfy its needs. In reality, it killed 6.26 and 3.38, and consumed 5.45 and 1.57 *L. (R.) luteola* and *P. acuta* individuals completely, respectively, daily. As the water bug consumed 1.57 *P. acuta* in contrast to 5.45 *L. (R.) luteola* completely, the possibility of selection of the prey individuals by *S. rusticum* prior to capture is very remote. If that were the case, there would have been no chance of victimization of *P. acuta* by *S. rusticum*. The results show that *S. rusticum* was reluctant to swallow the flesh of *P. acuta*. Therefore, it is not expected that the water bugs would spend energy unnecessarily to capture and handle *P. acuta*. In reality this did occur. Thus, it seems that the water bug was unable to recognize the prey individuals with respect to the species under reference. This again raises the question of the swallowing of the snail *P. acuta*. If *P. acuta* were captured by mistake, then it would be expected that the water bug would refuse the same, when it became known because of taste that the prey was not *L. (R.) luteola*. But we have cases where *S. rusticum* devoured the flesh of *P. acuta* completely. However, this was not a case of parallel choice of the prey individual *P. acuta* with respect to *L. (R.) luteola*, but more likely a feeding choice to satisfy hunger and ensure survival.

However, whatever the degree of preference for the prey snails, *L. (R.) luteola* and *P. acuta*, the water bug *S. rusticum* would prove effective in killing both prey species at an almost equal rate in a natural population, be it a single prey species population or a mixed population of both species. Therefore, consideration should be given to employing *S. rusticum* to control the snails *L. (R.) luteola* and *P. acuta* with a view to minimizing the hazards associated with these species (Macha, 1971; Raut, 1986; Subba Rao, 1989; Srivastava, 1991).

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#### Two Genera of North American Freshwater Snails: *Marstonia* Baker, 1926, Resurrected to Generic Status, and *Floridobia*, New Genus (Prosobranchia: Hydrobiidae: Nymphophilinae)

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Herein we recognize two genera of North American freshwater snails of the hydrobiid subfamily Nymphophilinae. One genus is resurrected from the synonymy of *Pyrgulopsis* Call & Pilsbry, 1886, while the other is newly proposed to accommodate species from the eastern United States previously placed in the genus *Cincinnatia* Pilsbry, 1891.

Baker (1926) proposed *Marstonia* as a subgenus of *Amnicola* Gould & Haldeman, 1840, containing *A. lusitrica* Pilsbry, 1890. Subsequently Baker (1928) added seven other species (all from northeastern North America) to this group, all of which are either currently placed in other genera or are fossils that are not readily assignable to genus. Berry (1943) showed that the penes of *Amnicola* and *Marstonia* differ in terms of internal ducts (and other features), and Morrison (1949) implied that these taxa should be placed in separate subfamilies of Hydrobiidae on this basis. Thompson (1970) redefined *Marstonia* and restricted it to the type species and one (new) species from the southeastern United States. Thompson (1977) subsequently expanded *Marstonia* to include six other eastern North American species which he described in detail. He noted the close morphological similarity between *Marstonia* and eastern species of *Pyrgulopsis*, but continued to recognize these as separate genera pending



study of the poorly known, extinct type species of the latter, *P. nevadensis* (Stearns, 1883). Hershler & Thompson (1987) studied resuscitated dried material of this western species, showed that its penis is closely similar to that of *Marstonia*, and synonymized the latter with *Pyrgulopsis* largely on this basis. However, a subsequent study showed that eastern North American species assigned to *Pyrgulopsis* are strongly differentiated morphologically from western congeners (Hershler, 1994) and has led us to re-evaluate the status of *Marstonia* and again recognize it as a distinct genus.

*Marstonia* Baker, 1926

*Marstonia* Baker, 1926:195.

**Diagnosis:** Eastern North American nymphophilines with ovate- to elongate-conic shells. The penis bears a small terminal lobe. The penial filament is variably sized. The penial ornament consists of a terminal gland and sometimes a ventral gland. *Marstonia* is distinguished from other nymphophilines in that the oviduct and bursal duct join well in front of the posterior pallial wall (Hershler, 1994:fig. 5C). *Marstonia* is further distinguished from *Pyrgulopsis* by the more coarsely pitted protoconch sculpture, incomplete inner shell lip across the parietal wall, banded (as opposed to diffuse) pattern of mantle pigmentation, narrowly vertical oviduct coil, and bursal duct largely or entirely imbedded in (as opposed to superficial to) the albumen gland (Hershler, 1994).

**Type species:** *Amnicola lustrica* Pilsbry, 1890 (original designation).

**Other species included:** *Marstonia agarhecta* Thompson, 1970; *Marstonia arga* Thompson, 1977; *Marstonia castor* Thompson, 1977; *Marstonia comalensis* (Pilsbry & Ferriss, 1906) (originally *Amnicola comalensis* Pilsbry & Ferriss, 1906); *Marstonia halcyon* Thompson, 1977; *Marstonia letsoni* (Walker, 1901) (originally *Amnicola letsoni* Walker, 1901); *Marstonia ogmorhapha* Thompson, 1977; *Marstonia olivacea* (Pilsbry, 1895); *Marstonia ozarkensis* (Hinkley, 1915) (originally *Pyrgulopsis ozarkensis* Hinkley, 1915); *Marstonia pachyta* Thompson, 1977; *Marstonia scalariformis* (Wolf, 1869) (originally *Pyrgula scalariformis* Wolf, 1869).

**Distribution:** Eastern North America from south-central Texas to the Atlantic Coastal Plain.

**Remarks:** As circumscribed herein, *Marstonia* includes species that Thompson (1977) previously allocated to the genus, and other eastern species that were previously placed in *Pyrgulopsis* (Hershler, 1994). We also transfer *Amnicola comalensis* to *Marstonia* based on our unpublished studies which show this species to conform morphologically to this genus.

*Cincinnatia* Pilsbry, 1891, was established as a sub-genus of *Amnicola* to include *Paludina cincinnatiensis*

Anthony, 1841 (a synonym of *Paludina integra* Say, 1821). Baker (1928) elevated *Cincinnatia* to full generic status, and Thompson (1968) expanded it to include 11 species which share a simple conical shell and a complex pattern of penial glandular ornament. Hershler & Thompson (1996) showed that the type species, *C. integra*, uniquely has two female bursal ducts; other species placed in *Cincinnatia* share a completely different female genitalic groundplan (Davis & Mazurkiewicz, 1985; Hershler & Thompson, 1996; Thompson, 2000; Hershler, unpublished data). Based on these observations, we restrict *Cincinnatia* to its type species and erect a new genus for other species from Florida and Maine. We propose that this genus be named for its center of diversity.

*Floridobia* Thompson & Hershler, gen. nov.

**Diagnosis:** Eastern American nymphophilines with ovate-conic shell. The penis has a large terminal lobe and a short filament. The penial ornament consists of large, crescent-shaped terminal and ventral glands, one or two narrow glands on the filament, and dorsal glands corresponding to Dg 1–3 (*sensu* Hershler, 1994). Additional glands on the dorsal and ventral surface are variably present and developed. *Floridobia* differs from all other North American nymphophilines in that females have a second, small anterior seminal receptacle (Thompson, 2000:figs. 23, 24). *Floridobia* further differs from *Cincinnatia* in that the dorsal glandular fields on the penis are not extensively fused; the bursa copulatrix is ovate or pyriform (but not cylindrical) and considerably overlaps the posterior end of the albumen gland; and there is only a single bursal duct which is superficial to or shallowly imbedded within the albumen gland.

**Type species:** *Amnicola floridana* Frauenfeld, 1863.

**Other species included:** *Floridobia alexander* (Thompson, 2000) (originally *Cincinnatia alexander* Thompson, 2000); *Floridobia fraterna* (Thompson, 1968) (originally *Cincinnatia fraterna* Thompson, 1968); *Floridobia helicogyra* (Thompson, 1968) (originally *Cincinnatia helicogyra* Thompson, 1968); *Floridobia leptospira* (Thompson, 2000) (originally *Cincinnatia leptospira* Thompson, 2000); *Floridobia mica* (Thompson, 1968) (originally *Cincinnatia mica* Thompson, 1968); *Floridobia monroensis* (Dall, 1885) (originally *Bythinella monroensis* Dall, 1885); *Floridobia parva* (Thompson, 1968) (originally *Cincinnatia parva* Thompson, 1968); *Floridobia petrifons* (Thompson, 1968) (originally *Cincinnatia petrifons* Thompson, 1968); *Floridobia ponderosa* (Thompson, 1968) (originally *Cincinnatia ponderosa* Thompson, 1968); *Floridobia porteri* (Thompson, 2000) (originally *Cincinnatia porteri* Thompson, 2000); *Floridobia vanhyningi* (Vanatta, 1934) (originally *Lyogyryus vanhyningi* Vanatta, 1934); *Floridobia wekiwae* (Thompson, 1968) (originally *Cincinnatia wekiwae* Thompson, 1968); *Flor-*

*idobia winkleyi* (Pilsbry, 1912) (originally *Amnicola winkleyi* Pilsbry, 1912).

**Distribution:** Eastern United States. Numerous species occur in Florida, while one congener (*F. winkleyi*) lives along coastal Maine.

**Etymology:** The name *Floridobia* (f.) is derived from the name of the State of Florida plus the Classical Greek βίος, meaning life. The name is feminine in keeping with the usual practice for diminutive creatures.

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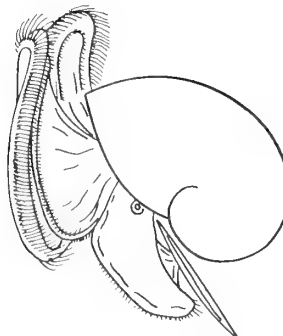
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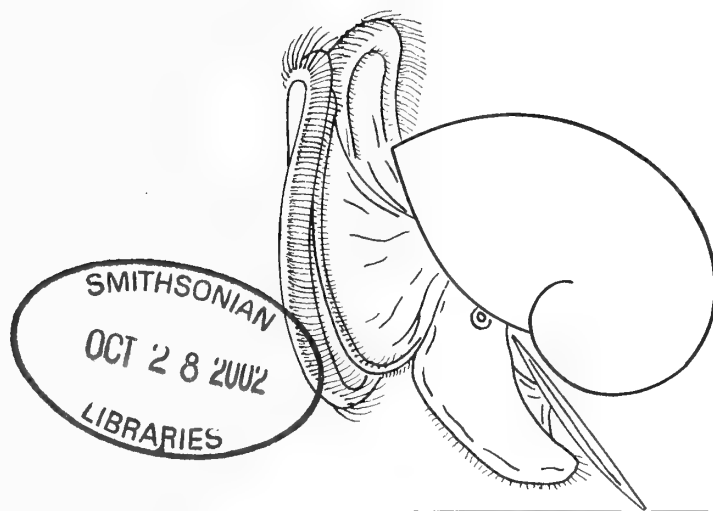
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## Movement and Wave Dislodgment of Mussels on a Wave-Exposed Rocky Shore

HEATHER L. HUNT\* AND ROBERT E. SCHEIBLING

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**Abstract.** Postlarval dispersal of mussels has the potential to greatly influence the dynamics of mussel assemblages on rocky shores. We individually tagged mussels (*Mytilus trossulus* Gould, *M. edulis* Linnaeus) in situ to compare rates of movement and loss between habitats (tidepools, emergent rock), positions (inside, outside of patches), and seasons. Between 7% and 86% of tagged individuals (5–25 mm shell length) moved  $\geq 1$  cm within 2–4 week intervals. Rates of movement were greater in July, when wave forces are lower, than in October, and were greater for mussels outside of patches than for those inside patches. Most tagged mussels moved distances of 1–2 cm, although 9% of movements were  $>10$  cm. Many of the tagged mussels disappeared over the course of the 3–4 week monitoring intervals, most likely due to wave dislodgment. The frequency of mussel disappearance was generally similar inside and outside of patches and between habitats, with the exception of a higher disappearance rate in October than July 1995 for mussels in tidepools but not on emergent rock. This study demonstrates that mussel patches on a wave-exposed shore are dynamic, with movements constantly rearranging individuals within patches, and high rates of loss of individuals, presumably from wave disturbance.

### INTRODUCTION

Mussels form patches or large beds on rocky shores, and often are major occupiers of space in the intertidal zone (Seed & Suchanek, 1992). Although mussels generally are thought of as sessile, they are not permanently attached to the substratum. Young postlarval mussels can use byssal threads that increase hydrodynamic drag to drift in the water column (Sigurdsson et al., 1976; De Blok & Tan-Maas, 1977). Larger juvenile and adult mussels may disperse actively over short distances by crawling, or passively over greater distances by wave dislodgment.

Active dispersal by crawling generally has not been considered important in prior studies of intertidal mussel assemblages, although *Mytilus edulis* Linnaeus, 1758, within subtidal aggregations have been observed to constantly move and reorient themselves (Dolmer et al., 1994; Anthony & Svane, 1995). Mussels may be less mobile in the intertidal zone of wave-exposed shores, where they must attach firmly to the substratum to withstand wave forces, than in the subtidal zone. Nevertheless, even movements over small distances could greatly influence rates of growth and mortality if they change a mussel's location within a patch or result in movement to a new patch. Mussels living in the center of groups generally experience reduced growth, but greater protection from

predation compared to individuals around the edge (Okamura, 1986). Living within an aggregation also shields mussels from hydrodynamic forces acting along the direction of flow (Denny, 1987) and is predicted to buffer individuals against rapid changes in temperature (Hel-muth, 1998). Studies have found a negative (Okamura, 1986; Newell, 1990; Svane & Ompi, 1993) or positive (Hunt & Scheibling, 2001a) relationship between mussel growth rate and patch size.

Larger displacements of adult mussels are likely to occur passively through dislodgment and redistribution by waves. Dislodgment by waves is a major cause of loss of mussels in the intertidal zone (e.g., Paine & Levin, 1981). Although some of the mussels dislodged by waves undoubtedly die, others probably are redistributed to new patches. Adult mussels have been observed colonizing cleared areas on rocky shores (Paine, 1974; Wootton, 1993). At our study site in Nova Scotia, we found that most mussel colonists were  $>2$  mm in shell length (Hunt & Scheibling, 1998b). Also, the greatest changes in mussel patch size usually occurred suddenly and often were associated with storms, suggesting that large mussels were dislodged and re-deposited by waves (Hunt & Scheibling, 2001a).

In this study, we quantified rates of movement and disappearance of tagged mussels on a wave-exposed shore in Nova Scotia, Canada. Rates of movement were compared between habitats (tidepools and emergent rock), positions (inside and outside of patches), seasons (summer and fall), and years. Mussels were tagged in situ to avoid disturbance of their attachment to the substratum.

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## METHODS

This study was conducted on an exposed rocky shore at Cranberry Cove (44°28'N, 63°56'W) near Halifax, Nova Scotia, Canada. The shore is composed of granite platforms and outcrops with occasional large boulders. There are numerous tidepools in irregular depressions along the shore, ranging from a few decimeters to over 10 m in maximum dimension. The shore is exposed to large southerly swells during fall and winter storms. Mussel assemblages at Cranberry Cove consist of a mixture of *Mytilus trossulus* Gould, 1850, and *M. edulis*: approximately 65–80% of mussels in tidepools and on emergent rock are *M. trossulus*, the remainder consist of *M. edulis* and hybrids of the two species (Hunt & Scheibling, 1998b). *M. trossulus* and *M. edulis* cannot be distinguished visually at the small size of the individuals at our study site. Most mussels are < 5 mm in shell length (SL); very few exceed > 20 mm (Hunt & Scheibling, 1998a).

We investigated the mobility of mussels by tagging individuals ~ 5 to 25 mm in shell length with numbered plastic bee tags (Steele & Brodie Ltd., Hampshire, England). These tags are small (2.6 mm diameter, 0.02 mm thickness) and lightweight (0.0014 g) and presumably have no measurable effect on movement of mussels. The same tags have been used to study swimming movements of juvenile scallops within a similar size range (Carsen et al., 1995). In August 1994, we tagged 15 individuals in each of two tidepools and two plots of emergent rock and monitored them for 2–3 weeks. In July and October 1995, we tagged 20 mussels in both a tidepool and an adjacent plot of emergent rock and monitored them for ~ 4 weeks.

The mussels were tagged in situ after temporarily draining the water from the tidepools. We dried one shell valve on each selected mussel, cleaned it with acetone, and affixed a tag using cyanoacrylate glue. Mussels < 5 mm were not tagged because their small size made it difficult to attach a tag without gluing the valves shut. Tagged mussels were grouped into two categories of initial position: in natural patches (at center or edge) and outside of them (alone or in a small group, or on top of the single layer of mussels in a patch). Mussels on top of a patch were considered to be outside because their frequencies of movement and disappearance were more similar to those of solitary mussels than to those in patches. We determined the location of each tagged mussel at 2–10 day intervals by measuring the distances between the mussel and two reference bolts drilled into the rock. We converted these distances to x and y coordinates and trigonometrically calculated the distance moved by a mussel between sampling dates from the coordinates for each date. These distances are minimum values, since mussels could have moved nonlinearly between sampling dates. We compared distances of movement between habitats, positions, and dates using ANOVA or t-tests, and the fre-

quencies of movement and disappearance of mussels using contingency tables (G-test).

## RESULTS

The tagged mussels were mobile, although they moved infrequently and for short distances. In August 1994 and July 1995, 21 to 56% of mussels in patches and 67 to 86% of those outside of patches moved within 13–27 days (Figure 1). In October 1995, only 7–10% of mussels in patches and 43–66% of isolated mussels moved within 30 days (Figure 1). The percentage of mussels that moved did not differ significantly between tidepools and emergent rock during each monitoring interval (August 1994: in patches,  $G_1 = 2.87$ ,  $P = 0.09$ ; outside,  $G_1 = 0.27$ ,  $P = 0.60$ ; July 1995: in patches,  $G_1 = 1.44$ ,  $P = 0.23$ ; outside,  $G_1 = 0.07$ ,  $P = 0.79$ ; October 1995: in patches,  $G_1 = 0.11$ ,  $P = 0.74$ ; outside,  $G_1 = 0.56$ ,  $P = 0.46$ ). Mussels outside of patches in both habitats were significantly more likely to move than those in patches (habitats pooled; August 1994,  $G_1 = 6.66$ ,  $P = 0.01$ ; July 1995,  $G_1 = 10.48$ ,  $P = 0.001$ ; October 1995,  $G_1 = 11.49$ ,  $P = 0.001$ ). In 1995, a higher percentage of mussels in patches moved in July than in October (habitats pooled,  $G_1 = 4.93$ ,  $P = 0.03$ ), when wave heights were much greater (Hunt & Scheibling 2001b). The percentage of mussels outside of patches that moved did not differ significantly between these dates (habitats pooled,  $G_1 = 1.16$ ,  $P = 0.28$ ).

Distances moved by tagged mussels were usually < 5 cm with a modal class of 1–2 cm, although six out of 68 individuals moved 10–49 cm (Figure 2). Distance moved during August 1994 and July 1995 did not differ significantly between tidepools and emergent rock (August 1994, pooled across plots:  $F_{1,22} = 1.20$ ,  $P = 0.29$ ; July 1995,  $F_{1,26} = 0.84$ ,  $P = 0.37$ ; October 1995, outside mussels (there was insufficient data to include mussels in patches in the analysis):  $t_7 = 0.49$ ,  $P = 0.64$ ), or between mussels in patches and those outside (August 1994:  $F_{1,22} = 0.009$ ,  $P = 0.93$ ; July 1995,  $F_{1,26} = 0.008$ ,  $P = 0.93$ ), and there was no significant interaction between habitat and position (August 1994:  $F_{1,22} = 0.93$ ,  $P = 0.35$ ; July 1995:  $F_{1,26} = 0.37$ ,  $P = 0.55$ ).

During each of the monitoring intervals, some tagged mussels were not relocated. These mussels probably were dislodged by waves and moved beyond our limited survey range of ~ 50 cm radius around their initial location. These disappearances were unlikely to have been tag losses because some tags from 1994 were still visible in 1995. Disappearances also were unlikely to have resulted from predation. Mussels eaten by the whelk *Nucella lapillus*, the only abundant predator of mussels at this site (Hunt & Scheibling, 1998a, 2001a), remained attached to the substratum and were identified by the presence of a drill hole. We have occasionally observed crabs at Cran-

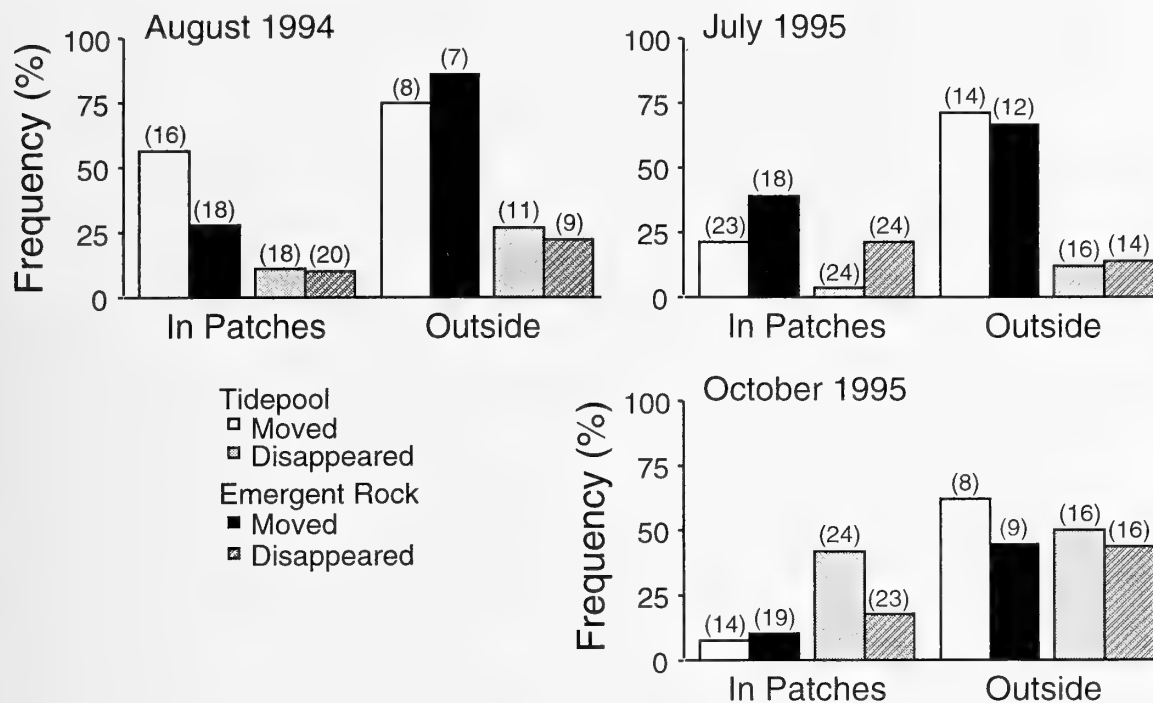


Figure 1. Frequency (%) of movement and disappearance of tagged mussels in patches and those outside of them (alone or in small group, or on top of the single layer of mussels in a patch) in tidepools and on emergent rock in August 1994 (pooled across plots within a habitat) and July and October 1995. Frequency of movement was calculated as a percentage of the mussels that were tracked throughout a monitoring interval. Frequency of disappearance was calculated as a percentage of the total number of tagged mussels. Sample size is indicated in parentheses.

berry Cove, but have found little evidence of crushed mussel shells indicative of crab predation.

In August 1994 and July 1995, 10–22% of mussels in patches and 13–27% of those outside of patches disappeared (Figure 1). In October 1995, when wave heights were greater, 42% and 17% of mussels in patches in tidepools and on emergent rock, respectively, and 44–50% of mussels outside of patches in both habitats, disappeared (Figure 1). The frequency of disappearance of mussels in July 1994 and August 1995 was too low to permit statistical comparisons of disappearance rate between habitats and positions. In October 1995, the frequency of disappearance did not differ significantly between tidepools and emergent rock, both for mussels in patches ( $G_1 = 3.40$ ,  $P = 0.065$ ), and for those outside of patches ( $G_1 = 0.13$ ,  $P = 0.72$ ). The frequency of disappearance also did not differ significantly between mussels in patches and those outside (habitats pooled,  $G_1 = 2.38$ ,  $P = 0.12$ ). In 1995, the frequency of disappearance in tidepools was significantly greater in October than in July, both for mussels in patches ( $G_1 = 10.8$ ,  $P = 0.002$ ) and those outside ( $G_1 = 6.5$ ,  $P = 0.011$ ). In contrast, the frequency of mussel disappearance on emergent rock did not differ significantly between dates for mussels in patches ( $G_1 = 0.14$ ,  $P = 0.710$ ) and those outside ( $G_1 = 3.2$ ,  $P = 0.07$ ). These results indicate that the frequency

of mussel disappearance was generally similar in and outside of patches and between habitats, with the exception of a higher disappearance rate in October than July 1995 for mussels in tidepools but not on emergent rock.

## DISCUSSION

It has long been recognized that *Mytilus edulis* detached from the substratum will crawl using their foot and byssal threads (e.g., Maas Geesteranus, 1942). However, most studies of mussels on hard substrates have recorded little mobility of undisturbed *Mytilus*. Our tagging study indicated that a significant proportion of mussels moved short distances. For example, 21–56% of mussels in patches in summer moved within 4 weeks. Some of these movements could have occurred by wave dislodgment rather than by crawling. In a study that examined mussels as a substrate for anemones, Anthony & Svane (1995) monitored movements of *M. edulis* in a subtidal mussel bed photographically. The frequency of movement of mussels in their study was higher (94% moved within 4 weeks) than in ours, possibly because lower water velocities in the subtidal permit mussels to be less strongly attached to the substratum. In contrast, Okamura (1986) found that *M. edulis* established in patches on tiles did not move from edge to central positions or vice versa. Paine (1974)

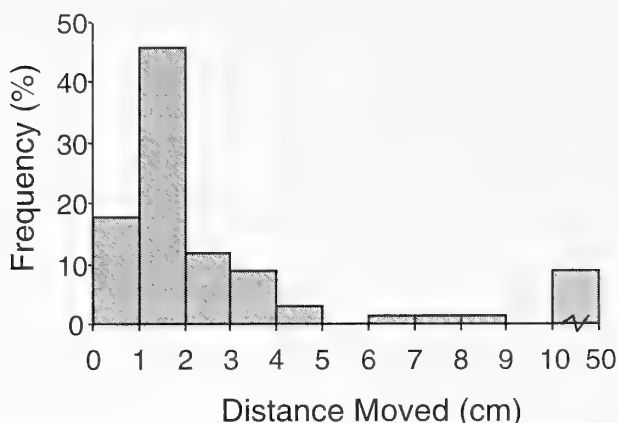


Figure 2. Frequency distribution of distances moved by tagged mussels. Mussels were pooled over habitats (tidepools, emergent rock), positions (in patch, outside) and dates (August 1994, July and October 1995). Sample size,  $n = 68$ .

found no distortion over 20 months of circles painted on undisturbed beds of *Mytilus californianus*, Conrad, 1837, although circles gradually became distorted if mussels were removed from an adjacent area. Mobility of mussels may depend on the availability of free space, the habitat type, and the species of mussel. Low mobility may be characteristic of *M. californianus*, the dominant mussel on the West Coast of North America. *M. californianus* crawls less rapidly (Harger, 1968), and has a stronger byssal attachment than other mytilids such as *Mytilus edulis*, *M. trossulus*, and *M. galloprovincialis* Lamarck, 1819 (Harger, 1970; Bell & Gosline, 1997).

In our study, mussels inside patches, which are bound by the byssal threads of their neighbors as well as their own attachment to the substratum, moved less frequently than mussels outside of patches. Movement rates of mussels within patches were two to three times higher in July than in October 1995, whereas movement rates of individuals outside of patches were similar in the two seasons. Byssal attachment of mussels varies seasonally (Price, 1980; Hunt & Scheibling, 2001b), and active movement by crawling may be easier in summer when byssal attachments are weaker (Hunt & Scheibling, 2001b) than in the fall.

The distances moved by tagged mussels in our study were generally small ( $< 10$  cm), resulting in changes in the position of a mussel within a patch or, less frequently, in emigration to a new patch (natural patches were usually separated by 5–15 cm). Distances of movement did not vary seasonally, between habitats, or between individuals inside or outside of patches. Movement within a patch may result in changes in growth rate or risk of predation, as these factors may vary with position in a patch (e.g., Okamura, 1986). Movement to a new patch may have similar consequences, as growth rate often de-

pends on patch size (Okamura, 1986; Newell, 1990; Svane & Ompi, 1993; Hunt & Scheibling, 2001a).

Many of the tagged mussels disappeared during the study (e.g., up to 27% of individuals tagged in summer). Although there may have been some tag loss, we believe that most of these individuals were dislodged by waves. Wave dislodgment is an important cause of disturbance for mussels on rocky shores (e.g., Paine & Levin, 1981). Loss rates of mussels in this study were consistent with probabilities of wave dislodgment calculated for mussels at Cranberry Cove using measurements of attachment strength and wave forces (Hunt & Scheibling, 2001b). The disappearance rate of mussels in tidepools was greater in October than July 1995, but did not differ between seasons on emergent rock. Dislodgment rates depend on both the wave forces imposed on individuals and on their attachment strength at a given time. Attachment strength and wave action vary seasonally, and these variations may counteract one another to dampen seasonality in the probability of wave dislodgment (Hunt & Scheibling, 2001b). At Cranberry Cove, wave forces are slightly higher on emergent rock than in tidepools (Hunt & Scheibling, 2001b). However, between-habitat differences in probability of dislodgment of mussels were predicted to vary over time as a result of variations in attachment strength. Some of the mussels dislodged by waves likely attach in new locations. We found that both juvenile and adult mussels immigrate into mussel patches (Hunt & Scheibling, 2001a) and colonize cleared areas (Hunt & Scheibling, 1998b).

In summary, we have documented movements of mussels in undisturbed patches on a wave-exposed shore. Detailed monitoring of individual mussels, such as that done in this study, can reveal small movements that otherwise may be overlooked. Such movements potentially have consequences for rates of growth and risk of predation of mussels, since these rates vary with patch size and position within a patch. We also measured rates of loss of mussels that were consistent with our previous predictions of rates of wave dislodgment at Cranberry Cove (Hunt & Scheibling, 2001b). These results, together with our previous work on colonization (Hunt & Scheibling, 1998b) and patch dynamics (Hunt & Scheibling, 2001a) of mussels at this site, demonstrate that postlarval dispersal can play an important role in the dynamics of mussel aggregations on rocky shores.

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## *Ankoravaratra*, a New Genus of Land Snails Endemic to Northern Madagascar (Cyclophoroidea: Maizaniidae?)

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**Abstract.** *Ankoravaratra*, gen. nov. has a simple shell resembling that of the East-African subgenus *Maizania* (*Micromaizania*) Verdcourt, 1964, from which it differs in having opercular coiling only half as tight. In reproductive morphology, *Ankoravaratra*, gen. nov. differs substantially from all anatomically known cyclophoroids, including maizaniids, so its familial placement remains uncertain. The genus contains five species, of which four are new and one is transferred.

### INTRODUCTION

This paper is one in a series reporting taxonomic results from the author's 1992–1996 survey and inventory of Madagascar's land mollusks (references in Emberton, 2002).

### MATERIALS AND METHODS

Materials were collected in 1995 using methods of Emberton et al. (1996). Identification and comparisons were made using Bequaert & Clench (1936), Wenz (1938–1944), Tielecke (1940), Morton (1952), Zilch (1959–1960), Solem (1959), Verdcourt (1963, 1964), Thompson (1969), Girardi (1978), Bruggen (1982, 1985, 1986, 1990), Fischer-Piette et al. (1993), and Emberton & Pearce (1999), and using the collections of the Florida Museum of Natural History. Templeton's (1989) cohesion concept was applied in delimiting species. Measurements were made using an ocular micrometer on a Wild M3C dissecting microscope. Dissections were on black wax under 70% ethanol, following procedures of Emberton & Pearce (1999: figures 32, 49, 50). Photographs were taken at standard magnifications (10× and 25×).

### LOCALITIES

Of the 1126 stations collected throughout Madagascar in 1992–1996, only the following 11 stations—all northern—yielded *Ankoravaratra*, gen. nov.

191–195. Montagne d'Ambre National Park, rainforest. 191. 12°35'S, 49°09'E, 1260 m, 11 July 1995. 192–195. 12 July 1995. 192. 12°35'S, 49°08'E, 1235 m. 193–194. 12°34'S, 49°09'E. 193. 1305 m. 194. 1280 m. 195. 12°31'S, 49°10'E, 1050 m.

201–213. Analamera Reserve. 201, 203. 12°44'S, 49°30'E. 201. 315 m, dry deciduous forest, 15 July 1995. 203–213. 16 July 1995. 203. 285 m, bamboo-dry decid-

uous thicket. 210, 213. 12°44'S, 49°29'E, dry deciduous floodplain forest. 210. 35 m. 213. 30 m.

256. South of Vohimar, 13°35'S, 49°59'E, 90 m, viny rainforest, 2 September 1995.

405, 407. Cap d'Ambre, Ambongoabo, 12°15'S, 49°15'E. 405. 320 m, baobab-deciduous forest, 25 August 1995. 407. 290 m, dry deciduous forest, 26 August 1995.

### SYSTEMATICS

Higher classification follows Ponder & Lindberg (1997; above superfamily) and Vaught (1989; superfamily and family). Latitudes and longitudes are given in degrees and minutes. To aid future workers, alcohol-preserved paratypes are listed separately. Types are placed in the Florida Museum of Natural History, University of Florida, Gainesville (UF); the Australian Museum, Sydney (AMS); the Academy of Natural Sciences of Philadelphia (ANSP); and the Muséum National d'Histoire Naturelle, Paris (MNHN, which does not assign catalogue numbers to types).

#### Class GASTROPODA

#### Clade CAENOGASTROPODA

#### Clade ARCHITAENIOGLOSSA

#### Superfamily CYCLOPHOROIDEA

#### Family MAIZANIIDAE?

#### *Ankoravaratra* Emberton, gen. nov.

(Figures 1–33)

**Type species:** *Ankoravaratra ambrensis*, sp. nov.

**Other species:** *A. ambalaniranae*, sp. nov.; *A. analamerae*, sp. nov.; *A. capdambrae*, sp. nov.; *A. imani*

(Fischer-Piette, Blanc, Blanc & Salvat, 1993), comb. nov.

**Diagnosis:** *Ankoravaratra*, gen. nov. has a simple shell and thin, horny, single-layered, nearly circular, spiral operculum resembling those of East-African subgenus *Maizania* (*Micromaizania*) Verdcourt, 1964, from which it differs in having opercular coiling only half as tight (opercular whorls equal to number of shell whorls versus double the number of shell whorls).

Anatomically, *Ankoravaratra* resembles *Owengriffithsius* (Emberton, 2002) in its bulbous-tipped penis in which seminal tube is enclosed (no seminal groove), apically looped, and subapically opening, and which bears a thick, semi-circular, flaplike gland; but *Ankoravaratra* has an extremely different shell morphology and has single-saccate (versus double-saccate) bursa copulatrix.

In penial morphology, *Ankoravaratra* differs substantially from all other (of the relatively few) anatomically known cyclophoroid genera, including maizaniids *Maizania* Bourguignant, 1889; *Maizaniella* Bequaert & Clench, 1936; and *Neomaizania* Bruggen, 1985.

Female reproductive system of *Ankoravaratra*, gen. nov. is drastically different from that of type species of *Maizania* in its unstalked (versus long-stalked) bursa copulatrix, absence (versus presence) of accessory sac on seminal receptacle, and relatively short and S-shaped (versus long and very convoluted) seminal receptacle.

Conchologically, *Theobaldius* G. Nevill, 1878, has similar operculum, shell shape and size, and smoothish sculpture to *Ankoravaratra*, gen. nov., but its peristome is more broadly reflected and is either doubled, or has a distinct anal notch, or both.

Other cyclophorid genera with similar operculum, such as *Cyclophorus* Montfort, 1810, have much larger, more robust shells with much thicker, more reflected peristomes.

*Ptychopoma* Möllendorf, 1885, has a somewhat similar shell, but its operculum is thick and calcified and its sculpture is not smoothish.

Other cyclophorid general that can be similar in shell shape and size, such as *Cyclotus* Swainson, 1840, and *Poteria* Gray, 1850, have extremely different opercula.

**Description:** Shell depressed-helicoid, diameter 6.4–9.6 mm, height/diameter 0.5–0.8, whorls 4.2–5.7, umbilicus/diameter 0.23–0.33. Spire low conic, sides of apex generally slightly concave. Body-whorl periphery rounded; suture deeply impressed, simple; whorl shoulders rounded. Aperture round; pre-apertural downward deflection slight, approximately 0.1 whorl. Apertural lip unreflected at upper suture, grading to partially and very narrowly reflected at umbilicus. Embryonic whorls 1.7–2.0; first 1.5 whorls 0.96–1.15 mm in diameter. Embryonic sculpture smooth. Body-whorl sculpture either smooth, with faint, irregular, axial growth lines; or with low, dense riblets. Color generally light, often with whitish flecks,

sometimes with single, reddish brown, peripheral color band.

Operculum fairly thin, horny, orange-yellow, broadly ovate, nearly circular, with parietal edge straight and rolled inward. Nucleus slightly eccentric toward baso-columnar edge. Whorls gradually and evenly increasing, approximately equal in number to shell whorls. On both external and internal surfaces, whorls bearing a low, broad, spiral ridge near suture. External surface smooth, glossy; internal surface rougher, with substructure resembling spirally radiating cross-laminated layers of parallel fibers.

Foot relatively short and broad, undivided. Snout short, divided into two lobes by narrow, central cleft. Testis large, nearly completely displacing apical digestive gland. Penis with completely enclosed seminal tube, without seminal groove. Penis cylindrical, apically swelling then tapering and coiling back on, and adhering to, itself in a 230–260° loose spiral. Penial pore thus subapical behind bulbous, false tip, and opening to side, but angled somewhat forward. Within penial pore, through translucent wall of true tip of the penis, a terminal, invaginable, intromittant portion of penis is visible. Left side of penis bearing a thick, semi-circular, flaplike gland that rolls partially around penial shaft. Ovary relatively small, lying along inside curve of apical digestive gland, consisting of tightly packed, bulbous acini. Oviduct (= “tube of FPSC” of Emberton & Pearce [1999]) with a sharp, V-shaped bend before running alongside, then tapering into, seminal receptacle. Seminal receptacle (= “albumen gland” of Thompson [1969] = “glandular base of FPSC” of Emberton & Pearce [1999]) narrow and V- to U-shaped shortly after its indistinct junction with oviduct, then swelling greatly and forming an S-shaped curve that straightens distally, before its junction with bursa copulatrix. Bursa copulatrix (= “seminal receptacle” of Thompson [1969] = “gland of FPSC” of Emberton & Pearce [1999]) a single, ductless sac.

**Etymology:** Malagasy “snail” (ankora) of the “north” (avaratra), for the strictly northern distribution of this genus in Madagascar.

**Gender:** Feminine.

#### Key to Species of *Ankoravaratra*:

- 1a. Diameter of first 1.5 whorls less than 1.00 mm ..... 2
- 1b. Diameter of first 1.5 whorls greater than 1.00 mm .... 3
- 2a. Diameter of first 1.5 whorls about 0.89 mm; inhabiting dry-deciduous forest on limestone karst ..... *imani*
- 2b. Diameter of first 1.5 whorls 0.96–0.98; inhabiting rainforest on non-calcareous base rock ..... *ambalaniranae*
- 3a. Body-whorl sculpted with low, dense riblets;



- body-whorl periphery faintly angulate; inhabiting rainforest ..... *ambrensis*
- 3b. Body whorl smooth, with only faint, irregular growth lines; body-whorl periphery round; inhabiting dry-deciduous forest ..... 4
- 4a. Diameter of first 1.5 whorls 1.10–1.15 mm; embryonic whorls 1.7–1.8; umbilicus broader, 0.29–0.32 shell diameter; shell generally with color band ..... *analamerae*
- 4b. Diameter of first 1.5 whorls 1.03–1.06 mm; embryonic whorls 1.9–2.0; umbilicus narrower, 0.23–0.27 shell diameter; shell generally without color band ..... *capdambrae*

### Species Descriptions

*Ankoravaratra ambalaniranae* Emberton, sp. nov.  
(Figures 25–29)

**Diagnosis:** Unique within genus for its combination of small initial-whorl size (diameter of first 1.5 whorls 0.96–0.98 mm) and rainforest habitat.

**Holotype:** Station 256 (UF 285436, 1 ad).

**Illustrated dry paratypes:** Station 256 (UF 285437, 3 ad, 1 juv).

**Other dry paratypes:** Station 256 (AMS C. 204777, 1 ad; ANSP 407915, 1 ad; MNHN, 1 ad; UF 285477, 4 ad, 7 juv).

**Type locality:** Madagascar, South of Vohimar, 13°35'S, 49°59'E, 90 m, viny rainforest.

**Description of holotype shell (Figure 25):** Female. Diameter 9.6 mm, height 6.5 mm, whorls 5.3, umbilicus 2.8 mm. Spire low conic, sides of apex slightly concave. Body-whorl periphery rounded; suture deeply impressed, simple; whorl shoulders rounded. Aperture round; height 3.6 mm, width 3.6 mm; downward deflection slight, 0.1 whorl. Apertural lip reflection grading from zero degrees at upper suture to about 60 degrees at umbilicus, narrow. Embryonic whorls 1.9; first 1.5 whorls 0.98 mm in diameter. Embryonic sculpture smooth. Body-whorl sculpture smooth, with faint, irregular, axial growth lines. Color light beige with white flecks. No color band.

**Shell variation:** See Table 1 and Figures 26–29.

**Operculum (Figure 29):** As for the genus.

**Etymology:** For Mount Ambalanirana, north of Sambava.

*Ankoravaratra ambrensis* Emberton, sp. nov.  
(Figures 1–19)

**Diagnosis:** Unique within the genus for its body-whorl sculpture of low, dense riblets and its faintly angulate body-whorl periphery.

**Holotype:** Station 191 (UF 285442, 1 ad).

**Illustrated dry paratypes:** Station 191 (UF 285443, 4 ad, 1 juv, 2 operc).

**Illustrated alcohol paratypes:** Station 191 (UF 285571, 6 ad [dissected]).

**Other dry paratypes:** Stations 191 (AMS C. 203496, 3 ad, 2 operc; ANSP 407916, 3 ad, 1 operc; MNHN, 3 ad, 1 operc; UF 285567, 28 ad, 63 juv); 192 (UF 285569, 3 ad, 3 juv); 193 (UF 285568, 5 ad, 8 juv); 194 (UF 285570, 2 ad); 195 (UF 285566, 1 ad).

**Other alcohol paratypes:** Stations 191 (UF 285572, 11 ad, 2 juv); 192 (UF 285573, 1 ad).

**Type locality:** Madagascar, Montagne d'Ambre National Park, 12°35'S, 49°09'E, 1260 m, rainforest.

**Description of holotype shell (Figure 1):** Female. Diameter 8.7 mm, height 5.7 mm, whorls 4.7, umbilicus 2.3 mm. Spire low conic, sides of apex slightly concave. Body-whorl periphery rounded, with just faint trace of angulation; suture deeply impressed, simple; whorl shoulders rounded. Aperture round; height 3.3 mm, width 3.4 mm; downward deflection slight, 0.1 whorl. Apertural lip reflection grading from zero degrees at upper suture to about 60 degrees at umbilicus, narrow. Embryonic whorls 1.8; first 1.5 whorls 1.09 mm in diameter. Embryonic sculpture smooth.

Body-whorl sculpture consisting of low, dense riblets, continuing into umbilicus. General color orangish beige with whitish flecks. Color band present, subperipheral, reddish brown.

**Shell variation:** See Table 1 and Figures 2–5.

**Operculum (Figures 6, 7):** As for the genus.

**Anatomy (Figures 8–19, ethanol-fixed and -preserved):** As for the genus.

**Etymology:** For Montagne d'Ambre (Amber Mountain) National Park.

*Ankoravaratra analamerae* Emberton, sp. nov.  
(Figures 20–24)

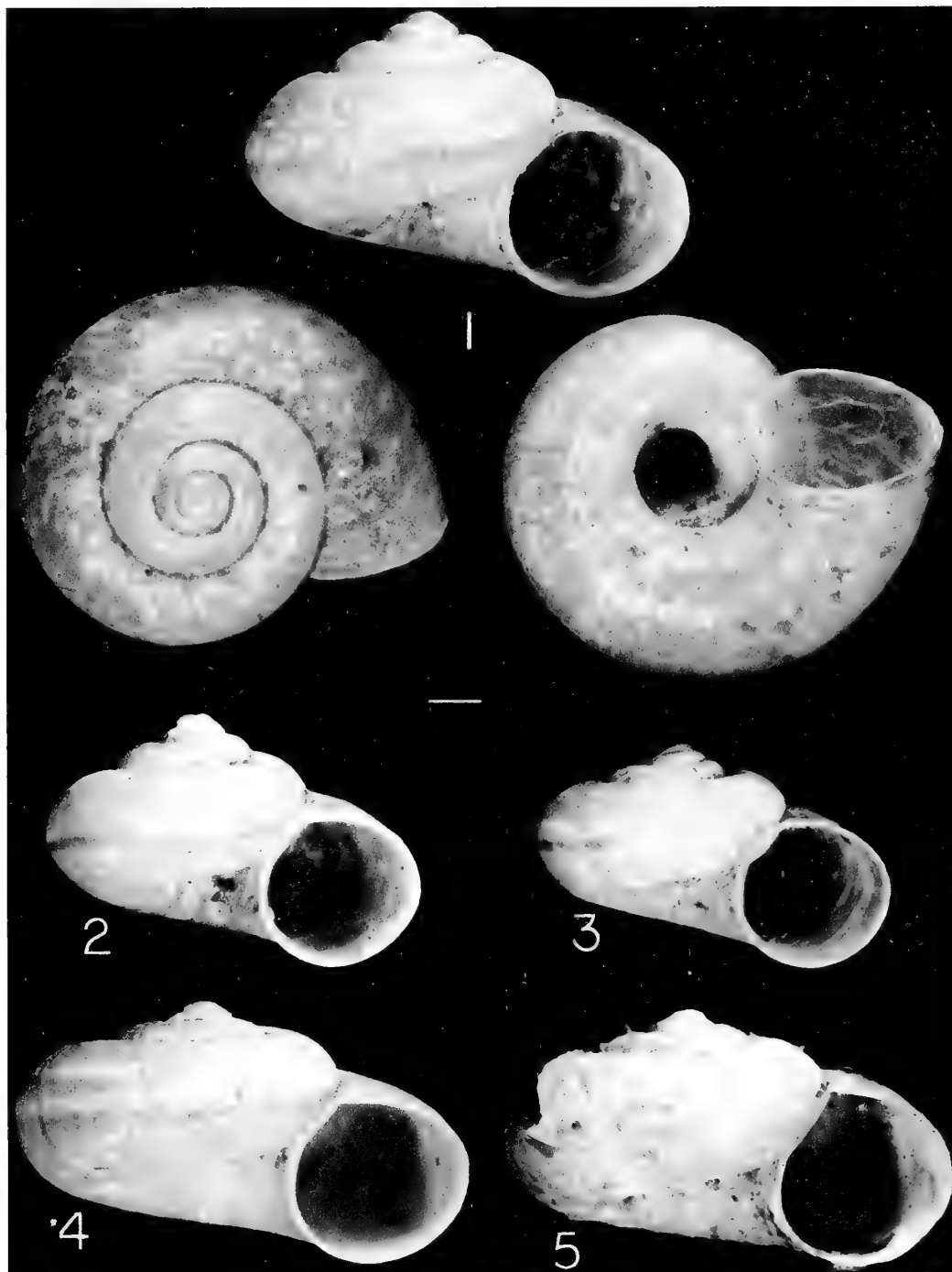
**Diagnosis:** Unique within the genus for its large initial whorl size (diameter of first 1.5 whorls 1.10–1.15 mm).

**Holotype:** Station 213 (UF 285438, 1 ad).

**Illustrated dry paratypes:** Stations 201 (UF 285440, 2 ad); 203 (UF 285441, 1 ad); 213 (UF 285439, 1 ad).

**Other dry paratypes:** Stations 201 (AMS C. 203497, 1 ad; ANSP 407917, 1 ad; MNHN, 1 ad; UF 285466, 7 ad, 1 juv); 203 (UF 285468, 6 ad, 4 juv); 210 (UF 285469, 1 ad, 1 juv); 213 (UF 285467, 8 ad, 5 juv).

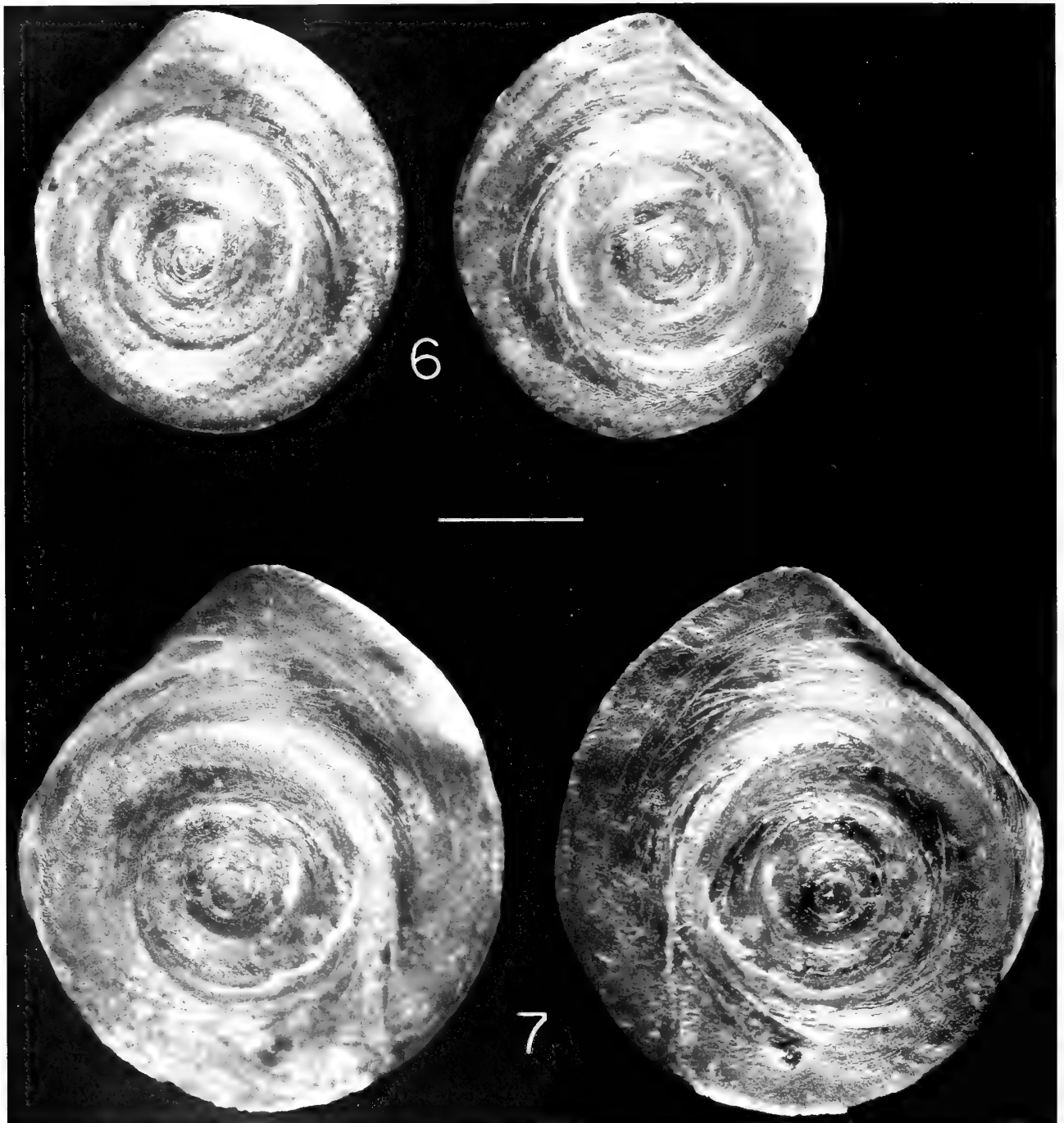
**Type locality:** Madagascar, Analamera Reserve, 12°44'S, 49°29'E, 30 m, dry deciduous floodplain forest.



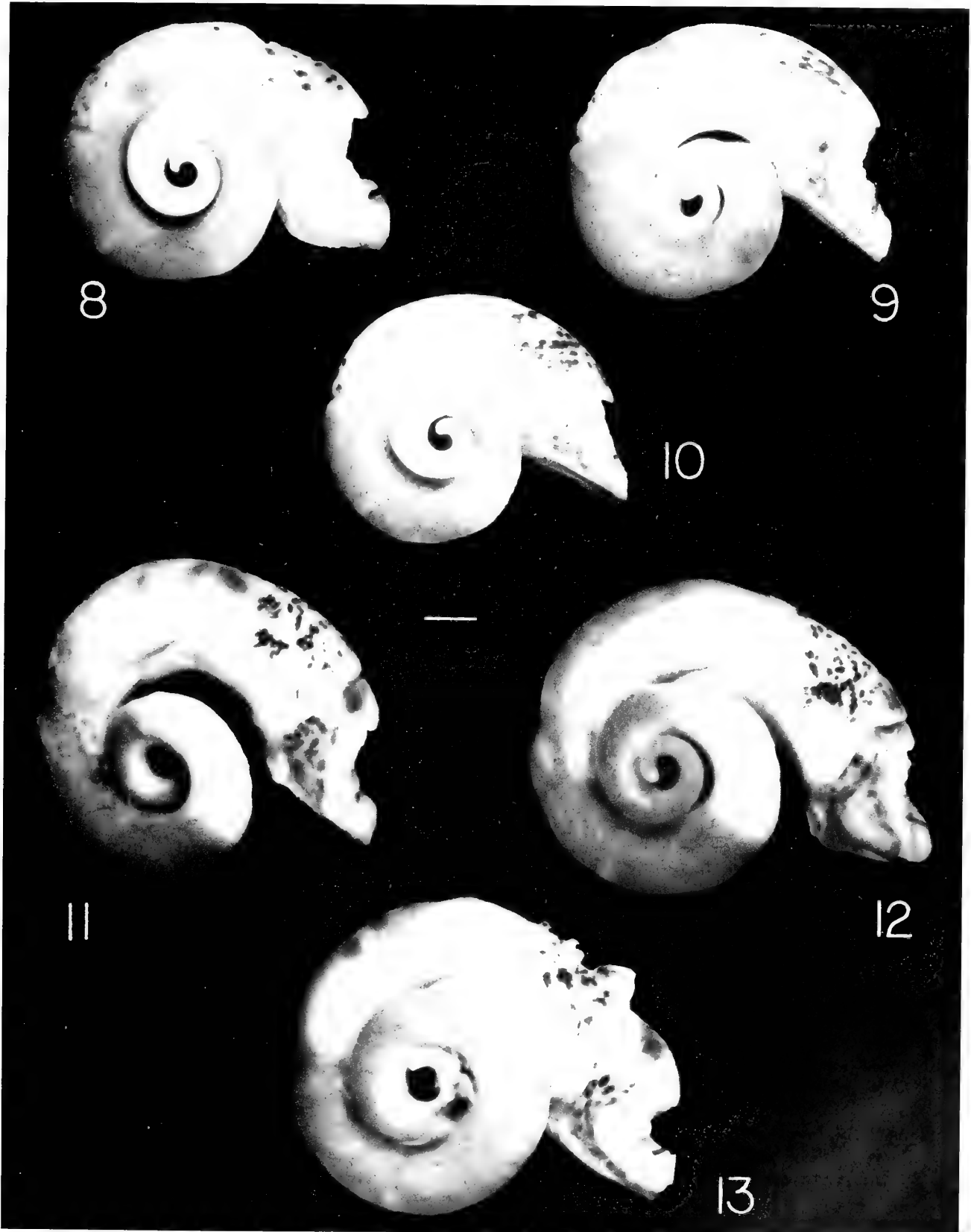
Figures 1–5. Shells of *Ankoravaratra ambrensis* Emberton, gen. & sp. nov. Figure 1. Holotype in three views (UF 285442). Figures 2–5. Paratypes from type locality, in one view (UF 285443). Figures 2, 3. Males, specimens #1, 2. Figures 4, 5. Females, specimens #3, 4. Scale bar = 1 mm.

**Description of holotype shell (Figure 20):** Female. Diameter 9.1 mm, height 6.3 mm, whorls 5.2, umbilicus 2.6 mm. Spire low conic, sides of apex slightly concave. Body-whorl periphery rounded; suture deeply impressed,

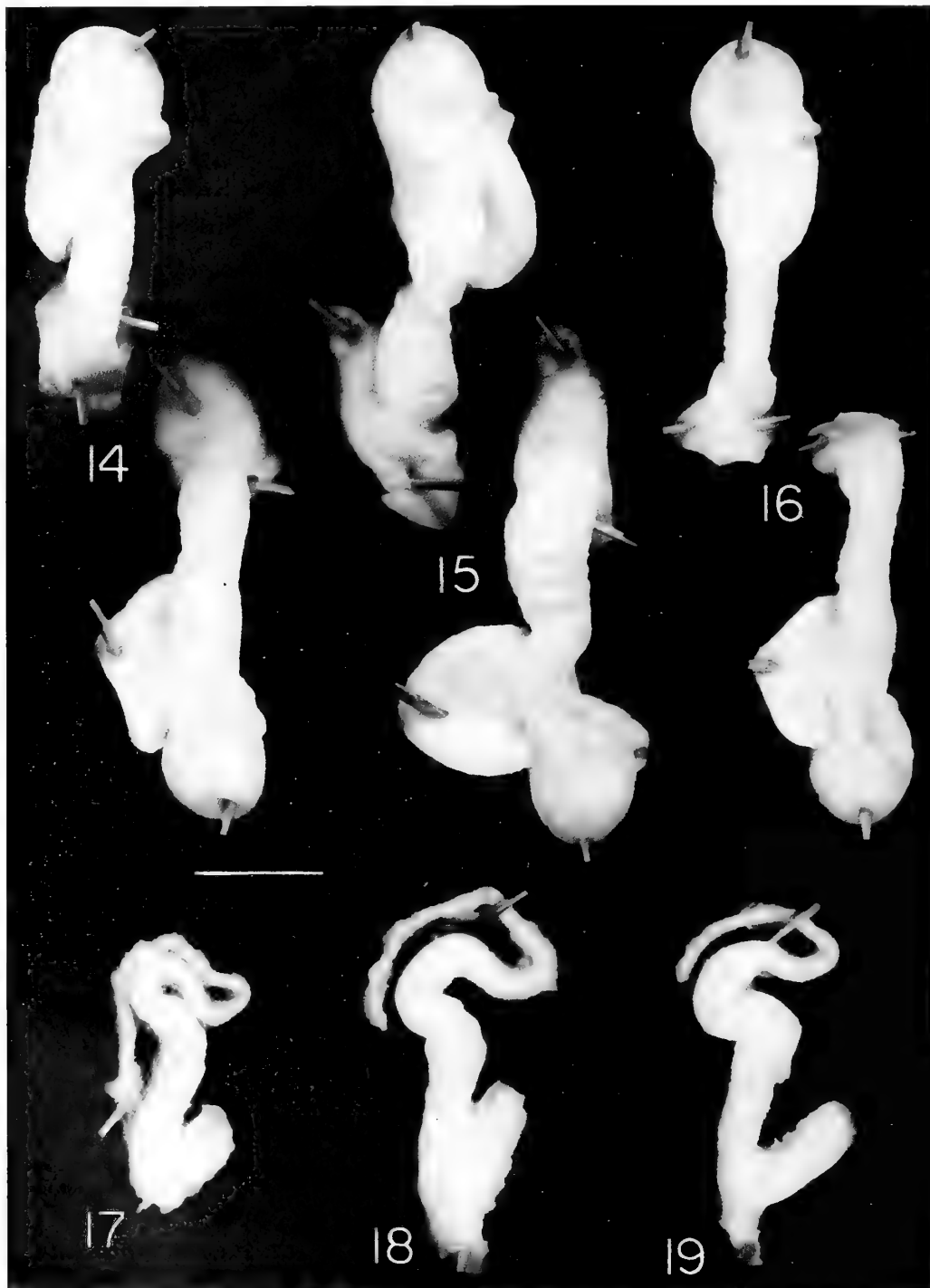
simple; whorl shoulders rounded. Aperture upright broadly oval; height 3.5 mm, width 3.4 mm; downward deflection slight, 0.1 whorl. Apertural lip reflection unknown, but reflected at umbilicus. Embryonic whorls 1.8;



Figures 6, 7. Opercula of *Ankoravaratra ambrensis* Emberton, gen. & sp. nov., in exterior (left) and interior (right) views (ex UF 285571). Figure 6. Type-locality male, specimen #3. Figure 7. Specimen #6. Scale bar = 1 mm.



Figures 8–13. Bodies (shells removed) of *Ankoravaratra ambrensis* Emberton, gen. & sp. nov., from the type locality (UF 285571). Figures 8–10. Males, specimens #1–3, respectively. Figures 11–13. Females, specimens #4–6, respectively. Scale bar = 1 mm.



Figures 14-19. Reproductive organs of *Ankoravaratra ambrensis* Emberton, gen. & sp. nov., from the type locality (UF 285571). Figures 14-16. Penes in dorsal and ventral views (upper and lower, respectively) of males, specimens #1-3, respectively. Figures 17-19. Oviduct-plus-seminal receptacle-plus-bursa copulatrix of females, specimens #4-6, respectively. Scale bar = 1 mm.

Table 1

Shell variation. Abbreviations: # specimen number, CBand color bands, D1.5W diameter of first 1.5 whorls, Dm shell diameter, EmW embryonic whorl count, fem female, Ht/D shell height divided by shell diameter, Um/D umbilicus diameter divided by shell diameter, W/lnD shell whorl count divided by natural logarithm of shell diameter (= index of coiling tightness), Whrl shell whorl count.

Species	Catalog #	#	Sex	Dm	Ht/D	Whrl	W/lnD	Um/D	D1.5W	EmW	CBand
<i>ambalaniranae</i>	UF 285436	—	fem	9.6	0.7	5.3	2.35	0.29	0.98	1.9	no
<i>ambalaniranae</i>	UF 285437	1	male	7.0	0.7	4.7	2.41	0.27	0.96	1.9	trace
<i>ambalaniranae</i>	UF 285437	2	male	7.2	0.7	4.8	2.44	0.31	0.98	1.9	yes
<i>ambalaniranae</i>	UF 285437	3	fem	9.6	0.7	5.1	2.26	0.30	0.98	1.8	no
<i>ambrensis</i>	UF 285442	—	fem	8.7	0.7	4.7	2.18	0.26	1.09	1.8	yes
<i>ambrensis</i>	UF 285443	1	fem	8.7	0.6	5.7	2.64	0.31	1.06	1.9	yes
<i>ambrensis</i>	UF 285443	2	fem	8.6	0.6	5.6	2.60	0.33	1.03	1.9	no
<i>ambrensis</i>	UF 285443	3	male	7.1	0.7	4.5	2.30	0.28	1.08	1.8	yes
<i>ambrensis</i>	UF 285443	4	male	6.7	0.7	4.4	2.33	0.29	1.06	1.9	yes
<i>ambrensis</i>	UF 285571	1	male	7.5	0.6	4.3	2.14	—	—	1.7	no
<i>ambrensis</i>	UF 285571	2	male	7.2	0.7	4.5	2.28	—	—	1.8	no
<i>ambrensis</i>	UF 285571	3	male	7.2	0.6	4.2	2.13	—	—	1.7	no
<i>ambrensis</i>	UF 285571	4	fem	8.3	0.6	4.7	2.23	—	—	1.7	no
<i>ambrensis</i>	UF 285571	5	fem	8.9	0.6	4.7	2.15	—	—	1.8	yes
<i>ambrensis</i>	UF 285571	6	fem	9.2	0.7	4.8	2.16	—	—	1.7	yes
<i>analamerae</i>	UF 285438	—	fem	9.1	0.7	5.2	2.35	0.29	1.10	1.8	yes
<i>analamerae</i>	UF 285440	1	male	7.7	0.5	4.3	2.12	0.32	1.13	—	yes
<i>analamerae</i>	UF 285440	2	fem	9.3	0.5	4.6	2.06	0.31	1.15	—	yes
<i>analamerae</i>	UF 285441	—	male	6.8	0.6	4.2	2.19	0.31	1.15	—	yes
<i>analamerae</i>	UF 285439	—	fem	8.5	0.6	4.7	2.20	0.29	1.10	1.7	trace
<i>capdambrae</i>	UF 285444	—	fem	9.1	0.8	5.1	2.31	0.23	1.06	1.9	no
<i>capdambrae</i>	UF 285445	—	male	6.7	0.7	4.6	2.43	0.27	1.03	2.0	no
<i>capdambrae</i>	UF 285446	1	fem	7.7	0.8	4.9	2.40	0.27	1.04	1.9	no
<i>capdambrae</i>	UF 285446	2	male	6.4	0.8	4.7	2.53	0.25	1.04	1.0	no

first 1.5 whorls 1.10 mm in diameter. Embryonic sculpture smooth. Body-whorl sculpture smooth, with faint, irregular, axial growth lines. General color orange-beige with purplish cast. Color band present, purplish brown, edged with white above and below.

**Shell variation:** See Table 1 and Figures 21–24.

**Etymology:** For Analamera Reserve.

*Ankoravaratra capdambrae* Emberton, sp. nov.

(Figures 30–33)

**Diagnosis:** Unique within the genus for its combination of large initial-whorl size (diameter of first 1.5 whorls 1.03–1.06 mm) and dry-deciduous-forest habitat.

**Holotype:** Station 407 (UF 285444, 1 ad).

**Illustrated dry paratypes:** Stations 405 (UF 285445, 1 ad); 407 (UF 285446, 2 ad).

**Other dry paratypes:** Stations 405 (UF 285472, 3 ad, 5 juv); 407 (AMS C.203498, 1 ad; ANSP 407918, 1 juv; MNHN, 1 ad; UF 285473, 1 ad, 4 juv).

**Type locality:** Madagascar, Cap d'Ambre, Ambongoabo, 12°15'S, 49°15'E, 290 m, dry deciduous forest.

**Description of holotype shell (Figure 30):** Female. Diameter 9.1 mm, height 7.1 mm, whorls 5.1, umbilicus 2.1 mm. Spire conic, slightly domed. Body-whorl periphery rounded; suture deeply impressed, simple; whorl shoulders rounded. Aperture upright broadly oval; height 3.4 mm, width 3.3 mm; downward deflection great, 0.2 whorl. Apertural lip reflection grading from zero degrees at upper suture to about 60 degrees at umbilicus, extremely narrow. Embryonic whorls 1.9; first 1.5 whorls 1.06 mm in diameter. Embryonic sculpture smooth. Body-whorl sculpture smooth, with faint, irregular, axial growth lines. General color brownish yellow, apex and upper whorls light orange. No color band.

**Shell variation:** See Table 1 and Figures 31–33.

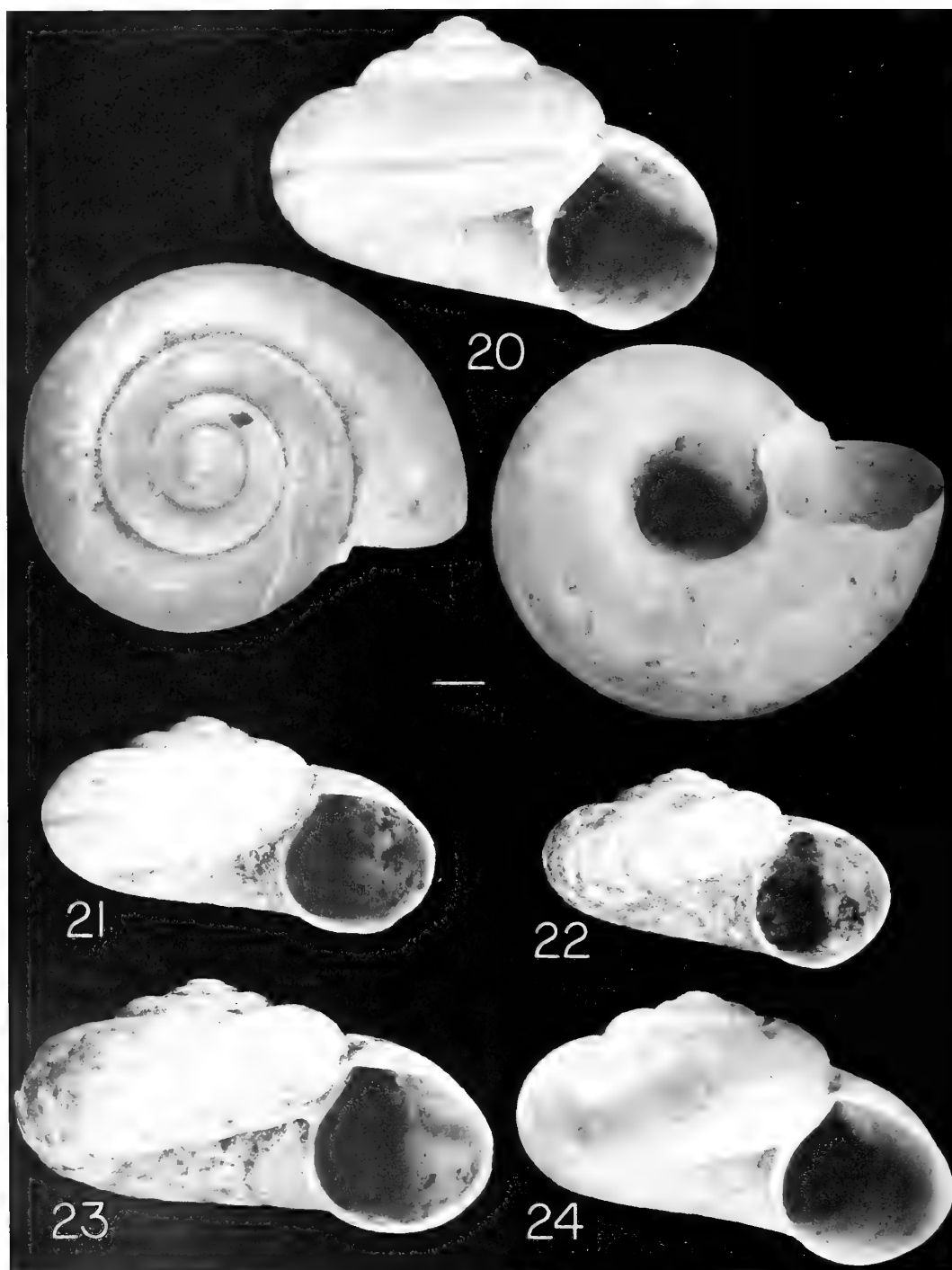
**Operculum (Figure 33):** As for the genus.

**Etymology:** For Cap d'Ambre (Tanjona Bobaomby).

*Ankoravaratra imani* (Fischer-Piette, Blanc, Blanc & Salvat, 1993), comb. nov.

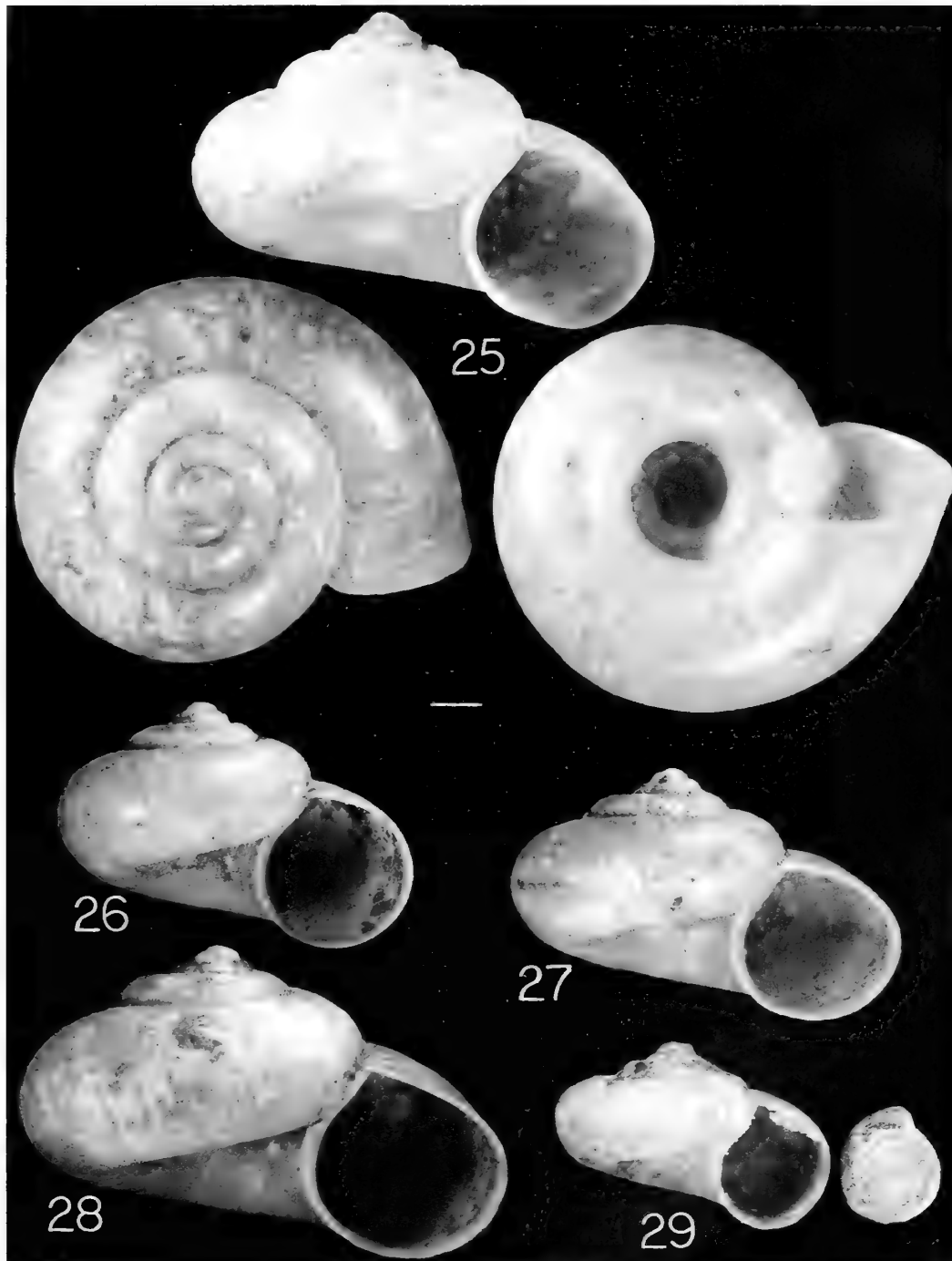
*Chondrocylus* (?) *imani* n. sp., Fischer-Piette et al., 1993: 17–19, figure 11.

**Diagnosis:** Unique within the genus for its very small initial whorl (diameter of first 1.5 whorls about 0.89 mm).



Figures 20–24. Shells of *Ankoravaratra analamerae* Emberton, gen. & sp. nov. Figure 20. Holotype in three views (UF 285438). Figures 21–24. Paratypes in one view. Figures 21, 22. Males (UF 285440, specimen #1; and UF 285441, respectively). Figures 23, 24. Females (UF 285440, specimen #2; and UF 285439, respectively). Scale bar = 1 mm.

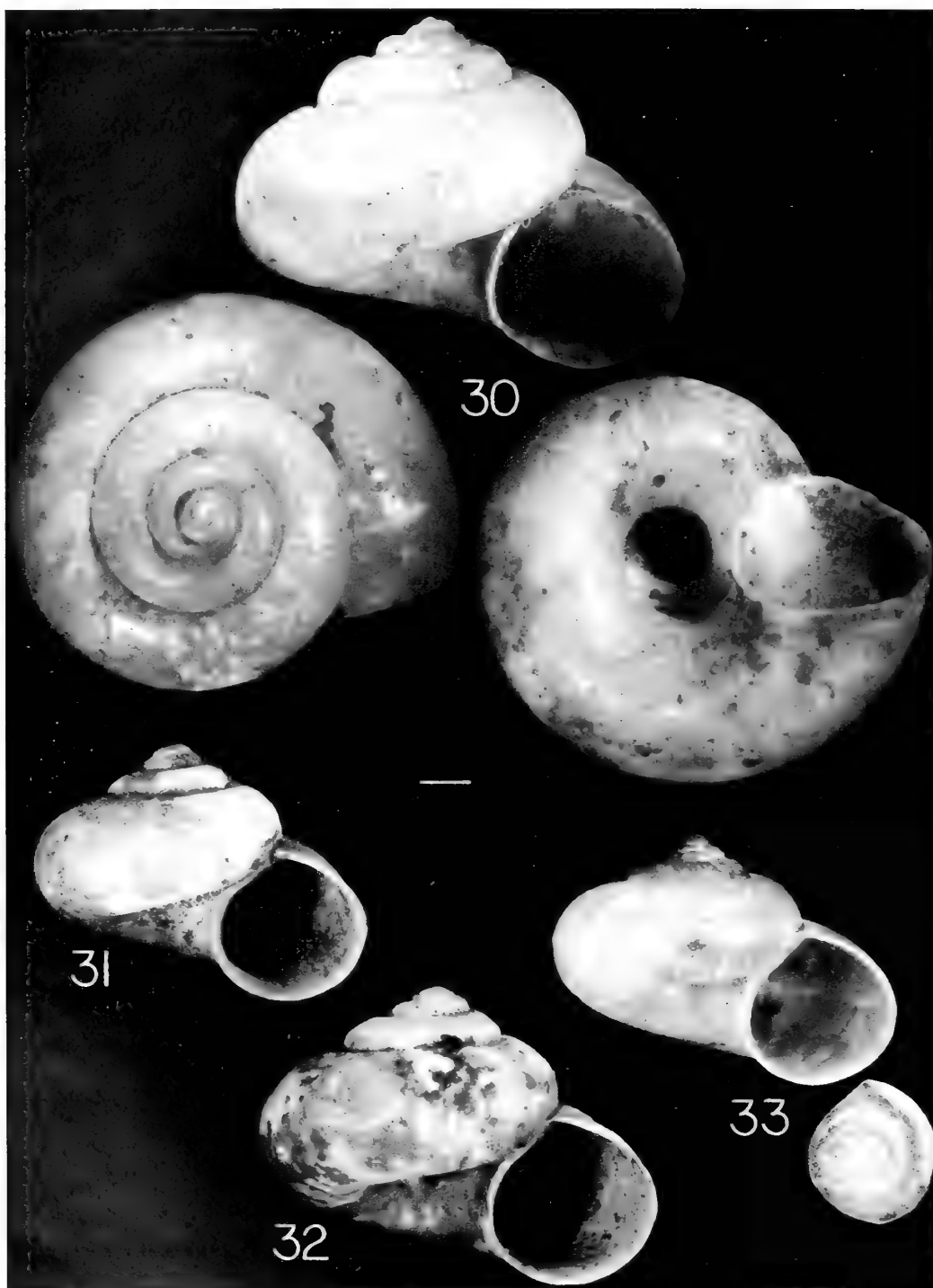




Figures 25–29. Shells of *Ankoravaratra ambalaniranae* Emberton, gen. & sp. nov. Figure 25. Holotype in three views (UF 285436). Figures 26–29. Paratypes from the type locality, in one view (UF 285437). Figures 26, 27. Males, specimens #1 and 2, respectively. Figure 28. Female, specimen #3. Figure 29. Juvenile with its operculum in interior view, specimen #4. Scale bar = 1 mm.

**Description of holotype shell:** Based on Fischer-Piette et al.'s (1993) figure 11. Diameter 7.1 mm, height 4.0 mm, whorls 4.7, umbilicus 2.4 mm. Spire low domed-conic, sides of apex slightly concave. Body-whorl pe-

riphery rounded; suture deeply impressed, simple; whorl shoulders rounded, apparently. Aperture nearly round; height 2.4 mm, width 2.5 mm; peristome reflection slight, narrow, greatest at columella. First 1.5 whorls approxi-



Figures 30–33. Shells of *Ankoravaratra capdambrae* Emberton, gen. & sp. nov. Figure 30. Holotype in three views (UF 285444). Figures 31–33. Paratypes in one view. Figures 31, 32. Male and female, specimens #2 and 1, respectively, from type locality (UF 285446). Figure 33. Male with its operculum in exterior view (UF 285445). Scale bar = 1 mm.

mately 0.89 mm in diameter. Embryonic sculpture smooth. Body-whorl sculpture of "very irregular growth lines, often located on the lower part of the whorl, without reaching the suture. Color "opaque, whitish, with the summit brownish-rosish." No color band.

**Distribution:** Ankarana Reserve, northern Madagascar.

**Acknowledgments.** Collection, sorting, housing, identification, and computer cataloguing funded by the U.S. National Science Foundation (DEB 9201060/9596032); description funded by Owen Griffiths. Permits were issued by the Madagascar government agencies DEF and ANGAP. Ranomafana National Park Project gave logistic support. Very many people collected or otherwise assisted, but particular thanks go to Dr. Tim Pearce, Jean Rakotoarison, the late Max Felix Rakotomalala, and Josephine Emberton. Dr. Fred Thompson and two anonymous reviewers gave useful comments on drafts of the manuscript.

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## Dichotomous Life History Patterns for the Nudibranch *Dendronotus frondosus* (Ascanius, 1774) in the Gulf of Maine

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**Abstract.** The nudibranch *Dendronotus frondosus* has a wide distribution and different morphological, ecological, and life history traits within its range. In the Gulf of Maine, populations can be found with either lecithotrophic or planktotrophic veliger larvae. Adults with these two types of larvae have overlapping habitat distributions, but the veligers differ in size, basic developmental characteristics, and composition of gelatinous clutches. Seasonal patterns of size distribution of adults suggest a sub-annual life cycle for those with planktotrophic larvae and an annual life cycle for those with lecithotrophic larvae. A feeding experiment with two types of hydroid prey resulted in lower growth rates for one dietary treatment, although this did not result in a shift in larval type. Mating recognition trials suggest a behavioral reproductive isolating mechanism between some populations. These results show little evidence for poecilogony and are motive for a taxonomic review of a *D. frondosus* complex in the Gulf of Maine.

### INTRODUCTION

Poecilogony is broadly defined as multiple larval development modes within members of a single species (Hoagland & Robertson, 1988; Levin & Bridges, 1995). Examples of this phenomenon have frequently been shown to be sibling species complexes (Hoagland & Robertson, 1988), and proven examples of poecilogonous marine invertebrates include a limited number of species of spionid polychaetes and opisthobranch gastropods (Levin, 1984; Bouchet, 1989; Krug, 1998). When studying differences in reproductive traits within these groups, the possibility for poecilogony must be examined.

*Dendronotus frondosus* (Ascanius, 1774) is a cosmopolitan nudibranch in northern temperate coastal waters and one of the most common opisthobranchs in the Gulf of Maine; however, descriptions of the habitat ecology, reproductive ecology, and general morphology of this species vary significantly (Alder & Hancock, 1845–1855; MacFarland, 1966; Robilliard, 1970). For example, a variety of hydroid diets have been associated with *D. frondosus* (McDonald & Nybakken, 1999). With this range of diets follow drastic differences in pigmentation patterns (Robilliard, 1975) and qualitative variation in ceras and foot morphology (personal observation). In addition, life history information varies considerably for reported reproductive season and general developmental characteristics (Swennen, 1961; Clark, 1975; Thompson & Brown, 1984). Because of these varying attributes, over 17 different taxonomic designations have confused the status of *D. frondosus*, as this species continues to be redefined in the literature (Robilliard, 1970; Tholleson, 1998). Reports of this species have been accumulated from Norway, Greenland, the western and eastern North

American coasts, and the northern Asiatic coast (Robilliard, 1970), largely because this species epithet continues to be a general designation for the genus *Dendronotus*.

*Dendronotus frondosus* feeds on a variety of athecate (e.g., *Tubularia* spp.) and thecate (e.g., *Obelia* spp.) hydroids (Miller, 1961; Swennen, 1961; Todd, 1981), and differences in diet often are coupled with drastic differences in physical habitats and seasonal population fluctuations. In the Gulf of Maine, *D. frondosus* habitats range from southern coastal areas associated with subtidal hydroid communities (Clark, 1975; Lambert, 1991) to the Bay of Fundy and northern Nova Scotia (Meyer, 1971; Bleakney, 1996). Throughout the region these slugs are commonly found in subtidal thecate hydroid communities and fouling communities with athecate hydroid colonies (Meyer, 1971; Clark, 1975; Lambert, 1991). In northern regions of the Gulf of Maine, *D. frondosus* regularly occurs in intertidal habitats associated with the thecate hydroid *Sertularia pumila*, a common rockweed epiphyte (Meyer, 1971; Gionet & Aiken, 1992; Bleakney, 1996).

In the North Atlantic, in addition to variability of habitats, *D. frondosus* shows a range of reproductive patterns. Reports of seasonal spawning vary from strictly annual (Clark, 1975) to nearly year-round (Swennen, 1961). Larval feeding type may also differ; both lecithotrophic (Thompson & Brown, 1984) and planktotrophic larvae (Clark, 1975) have been described for this species on opposite sides of the Atlantic. Hoagland & Robertson (1988) noted that these allopatric differences in life history data warrant further examination for the possibility of poecilogony.

This paper outlines subtidal and intertidal habitats of *D. frondosus* in the Gulf of Maine and describes variation in the larval development and feeding type of this nudi-

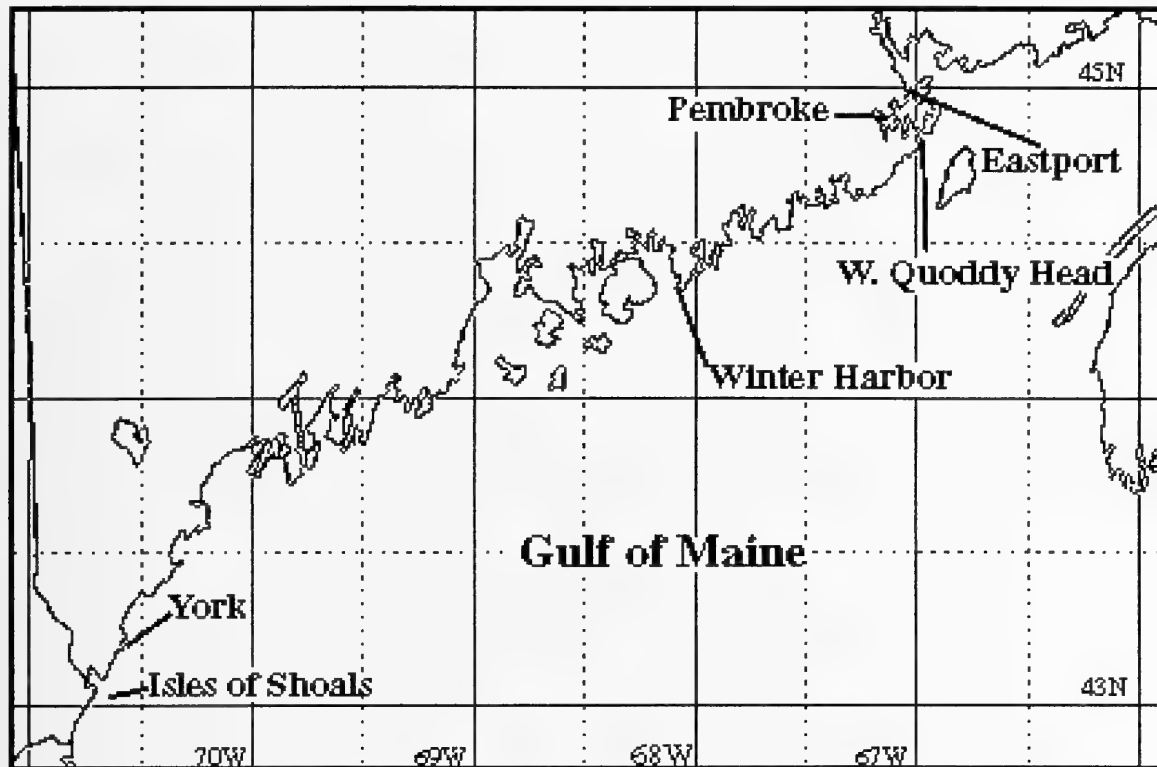


Figure 1. Map of study sites on the Maine and New Hampshire coastline. Southern subtidal sites include York and Isles of Shoals. Northern subtidal sites include Winter Harbor, Eastport, and Pembroke. Northern intertidal sites include Pembroke and West Quoddy Head.

branch associated with differences in habitat ecology. These observations were motive for a simple reciprocal feeding experiment to evaluate the effects of two major diets on the type of larvae produced. A mating recognition experiment provided data on the potential for reproductive isolation of the most disparate groups of *D. frondosus*, although a formal taxonomic review is not included in this paper.

## MATERIALS AND METHODS

### Collection and Life History Observations

I collected *Dendronotus frondosus* individuals from intertidal and subtidal (5–10 m deep) sites in the Gulf of Maine between March 1997 and August 1998 (Figure 1). Southern subtidal sites were at Cape Neddick in York, Maine (43°00'N, 70°36'W), and at the Isles of Shoals, New Hampshire (44°21'N, 68°03'W). Northern subtidal sites were at Winter Harbor (44°53'N, 67°09'W), Eastport (44°54'N, 66°59'W) and Pembroke (44°53'N, 67°09'W), Maine. Northern intertidal collection sites included Pembroke and West Quoddy Head (44°49'N, 66°57'W), Maine. I monitored these sites three to six times/year to establish seasonal patterns in spawning behavior. Adult *D. frondosus* were maintained in a 10°C temperature-con-

trolled room at the University of New Hampshire (UNH) Durham campus and fed hydroids (*Obelia* spp. and *Sertularia* spp.) found as epiphytes on the rockweed *Ascophyllum nodosum*. Lengths of nudibranchs were measured with an ocular micrometer and a dissecting microscope while the animals were actively crawling on a flat submerged surface.

I isolated individual nudibranchs for 1–2 days at 10°C in order to collect spawn masses when they were deposited. All larval measurements were made with an ocular micrometer ( $\pm 10 \mu\text{m}$ ) and a compound microscope. Zygote diameters were measured by haphazardly selecting 10 zygotes in the center region of different spawn masses. The embryos were then placed in 200 mL plastic-covered containers with natural seawater (32–35 ppt) and gently aerated. I changed the water and culture container two times per week and monitored the development of the embryos until they began to hatch from the capsules and gel matrix of the mass. The number of days until hatching for each spawn mass ( $\pm 1$  day) was recorded. I then measured the maximum shell length from the aperture just above the velum of three to seven larvae from four spawn masses for each larval type. Immediately after hatching, several larvae were presented with hydroid material (*Obelia* and *Sertularia* spp.) and observed for signs of

induction of metamorphosis such as resorption of the velum and the loss of shell and operculum (Todd, 1981). Planktotrophic and lecithotrophic larvae from four different spawn masses for each type were placed into glass stacking dishes in seawater at approximately one to two veligers/mL. The larvae were maintained at 10°C and fed small amounts of the cultured microalgae, *Isochrysis galbana* and *Rhodomonas salina*. After 1 week of culture, the larvae were examined with a compound light microscope for the presence of algal material in their gut.

### Reciprocal Feeding Experiment

Two reciprocal feeding experiments helped me determine the quality of two hydroid diets and the plasticity of the larval types produced by adults fed these diets. I haphazardly collected 60 juvenile *D. frondosus* (12.9–34.5 mm) from northern intertidal sites in October 1997, and placed them in covered plastic containers (18.4 × 16.5 × 11.1 cm) with two opposite mesh windows in flow-through seawater tables at the UNH Coastal Marine Laboratory in Newcastle, New Hampshire. The two dietary treatments were either *Obelia* or *Sertularia* spp. found as epiphytic colonies on *Ascophyllum nodosum* on a nearby pier, fed to the nudibranchs one to two times per week, amounting to an *ad libitum* regime. After 3 months, I measured the remaining 51 individuals (22 fed on *Obelia* spp. and 29 fed on *Sertularia* spp.) and paired them for mating and production of spawn masses. Eleven pairs of nudibranchs from the *Obelia* treatment and 13 pairs fed *Sertularia* spp. were observed as they spawned for 2 months.

A similar experiment used *D. frondosus* from southern subtidal sites with the same feeding and maintenance schedules. Starting lengths ranged from 3.3–16.7 mm for the 72 juveniles collected between March and April 1998. The final measurements were collected after only 4 weeks of growth when mortality reduced the number of survivors to 36 slugs (24 fed *Obelia* spp., 12 fed *Sertularia* spp.). Additional mortality reduced the number of pairs of nudibranchs to 10 pairs for the *Obelia* treatment and five pairs for the *Sertularia* treatment which were observed as they spawned for an additional 2 weeks.

The total number of spawn masses was then recorded and the larvae were allowed to develop until I could distinguish them as either planktotrophic or lecithotrophic larvae. The assessment of these larval types was based on morphological characteristics of the digestive gland, propodium, and shell. Growth data were analyzed using a 2-tailed t-test, and the number of spawn masses between treatments was compared using a Mann-Whitney test for non-parametric data.

### Mating Recognition Behavior

Adult slugs between 1.3 and 4.1 cm long were collected in May 1998 from breeding populations at northern

intertidal and southern subtidal sites. I isolated individuals in covered plastic containers (18.4 × 16.5 × 11.1 cm) at 10°C for at least 5 days prior to the experiment. Three treatment groups consisted of 18 pairs of similarly sized slugs (±1.0 cm). Pairs of adults that were both collected from northern intertidal sites composed one treatment group, another used pairs from southern subtidal sites, and the experimental group consisted of one adult *D. frondosus* from each of the two habitats. I checked the pairs every hour during a 7-hour period on the first day and a 4-hour period for another 4 days and recorded the number of pairs engaged in copula. Coupling lasted between 2–7 hours, so this monitoring routine was adequate for recording these mating events. I compared frequency counts of mating between the mixed pairs and either the northern intertidal or southern subtidal pairs using a 2 × 2 contingency table with a Log-likelihood ratio (G-test) and a Yates correction for continuity. In addition, I paired four adults from northern subtidal sites: two with individuals from northern intertidal sites and another two with adults from southern subtidal sites. After observing for mating recognition, I then switched pairings to document the behavior with adults from the other sites (either northern intertidal or southern subtidal).

## RESULTS

### Habitats and General Characteristics

*Southern Subtidal Habitats.* *D. frondosus* is found associated with colonies of *Obelia* spp. growing on rocky ledges and as epiphytes on the kelps *Laminaria* spp. and *Agarum cribosum* (Lambert, 1991). Other nudibranchs common to these communities include *Coryphella verrucosa*, *Tergipes tergipes*, and *Doto coronata*. *D. frondosus* at these sites produces only spawn masses with developing planktotrophic larvae. Adult *D. frondosus* commonly had extensive white or dark brown mottling on a reddish brown body.

*Northern Intertidal Habitats.* *D. frondosus* is found midway through the protected rockweed zone in dense beds of *Ascophyllum nodosum* (Gionet & Aiken, 1992; Bleakney, 1996). Here, these slugs are commonly found eating the epiphytic and epilithic hydroid *Sertularia pumila*. The only other nudibranch frequently found in these habitats is a bryozoan feeder, the dorid *Acanthodoris pilosa*. *D. frondosus* is found in rocky crevices at the base of *A. nodosum*. They deposit spawn masses on the algae and primary substrate, frequently intertwined with the hydroid colonies. Their color is strictly pale white-yellow, with extremely limited mottling on the dorsal side adjacent to the cerata. More colorful *D. frondosus* with extensive mottling can be found infrequently in the littoral zone in southern regions of the Gulf of Maine such as Appledore Island, Maine, but never with the consistency and relatively high densities of those sites in the northern Gulf of Maine. Northern intertidal sites at West Quoddy

Head and Wilbur Neck (Figure 1) always had *D. frondosus* spawn masses that yielded lecithotrophic larvae. No planktotrophic larvae were found in *D. frondosus* masses at these habitats.

**Northern Subtidal Habitats.** I found *D. frondosus* at subtidal sites in Winter Harbor, Eastport, and Pembroke, Maine (Figure 1) associated with *Obelia* spp. and related thecate hydroids. These slugs were either reddish brown with white and dark mottling or pale purple or white with limited or no mottling. Collected spawn masses produced either lecithotrophic or planktotrophic larvae depending on the time of year of sampling.

### Seasonal Spawning and Adult Size Distribution

At two southern subtidal sites, York and the Isles of Shoals, *D. frondosus* had an annual spring spawning event with adults that, when present, were producing spawn masses (Figures 2a, b). After spawning, adult populations senesced and for several months did not occur at these sites (Figures 2a, b). At two northern intertidal sites, West Quoddy Head and Wilbur Neck, *D. frondosus* also had an annual spawning period in the spring or early summer (Figures 2c, d); however, new recruits appeared at these sites a few months after the spawning event and gradually increased in size throughout the fall and winter without depositing spawn masses apparently until the following spring (Figures 2c, d). Note that there was a brief annual period at the Wilbur Neck site when no *D. frondosus* were found (Figure 2d). The two northern intertidal sites were offset in the timing of these patterns of growth and spawning, with Wilbur Neck having an earlier spawning event than West Quoddy Head (Figures 2c, d).

### Larval Types and Characteristics

No mixed clutches (i.e., both planktotrophic and lecithotrophic larvae) were found at any of the collection sites. All spawn masses from the two types of *D. frondosus* had one embryo/capsule and each formed a hollow cylindrical capsule-filled cord that was attached along one side, or Type B according to Hurst (1967). All veliger larvae had Type 2 inflated egg-shaped shells (Thompson, 1961).

There were large differences in the zygote size, time spent in the embryonic capsule from deposition to hatching, and in larval shell size at hatching (Table 1). Lecithotrophic larvae began with relatively large zygotes and took approximately a month to hatch. When they did hatch, they were much larger than the planktotrophic larvae with a robust velum, a propodium and a visceral mass that occupied a large amount of the larval shell (Figure 3a). When offered microalgae, none were ingested by these veligers or at least were not immediately present in the larval gut. The lecithotrophic veligers began metamorphosis within a few hours to 1 day of hatching either on the egg mass material or on the hydroids *Obelia* spp.

and *Sertularia* spp. They were never observed metamorphosing within the embryonic capsule.

Planktotrophic veligers originated from smaller zygotes, took less time to hatch (approximately 1 week), and were much smaller upon hatching than their lecithotrophic counterparts (Table 1). These veligers would not metamorphose upon hatching despite the presence of hydroid material. They actively ingested microalgae when offered it, which was apparent by the presence of red and brown material in the larval gut and digestive gland. The planktotrophic larvae had a shell that was relatively unfilled by larval tissues such as the digestive gland and gut (Figure 3b), and the velum was frequently small with a minimal propodium (Figure 3b).

### Reciprocal Feeding Experiment

Both the northern intertidal and southern subtidal slugs showed less increase in growth on a diet of *Sertularia* spp. (Figure 4) than on *Obelia* spp. Although the starting lengths were similar for both trials, the final lengths were much higher for those fed *Obelia* spp. (Figures 4a, b) and the percent change in lengths was significantly higher for northern intertidal and southern subtidal individuals in this treatment. Similarly, the mean number of spawn masses produced per nudibranch was consistently less for all those fed *Sertularia* spp. (Figure 4d). The only significant differences for spawn mass output were found for the northern intertidal trial because of high levels of mortality in the southern subtidal treatment group (Figure 4d). Despite these differences in diet quality, all northern intertidal nudibranchs produced spawn masses yielding viable lecithotrophic veligers, and all southern subtidal *D. frondosus* had planktotrophic veligers. Once again, no mixed clutches were deposited. The characteristics presented earlier (Figure 3, Table 1) were used to distinguish these two larval types.

### Mating Recognition Behavior

Adult *D. frondosus* from northern intertidal and southern subtidal habitats did not recognize each other as potential mates (Table 2). These slugs did not engage in copula even though others individuals collected at the same sites were actively mating (Table 2). Since only planktotrophic larvae were produced by *D. frondosus* from southern subtidal sites and only lecithotrophic larvae came from those at northern intertidal sites, these crosses reflect the two developmental types. Sperm storage from previous mates cannot be ruled out in these trials; therefore, these data do not evaluate fertilization success. These results only represent the potential for behavioral recognition of adult mates. The limited number of trials with *D. frondosus* from northern subtidal sites suggests that these individuals mate exclusively with either the northern intertidal or the southern subtidal adult nudibranchs. None of the four adults from the northern



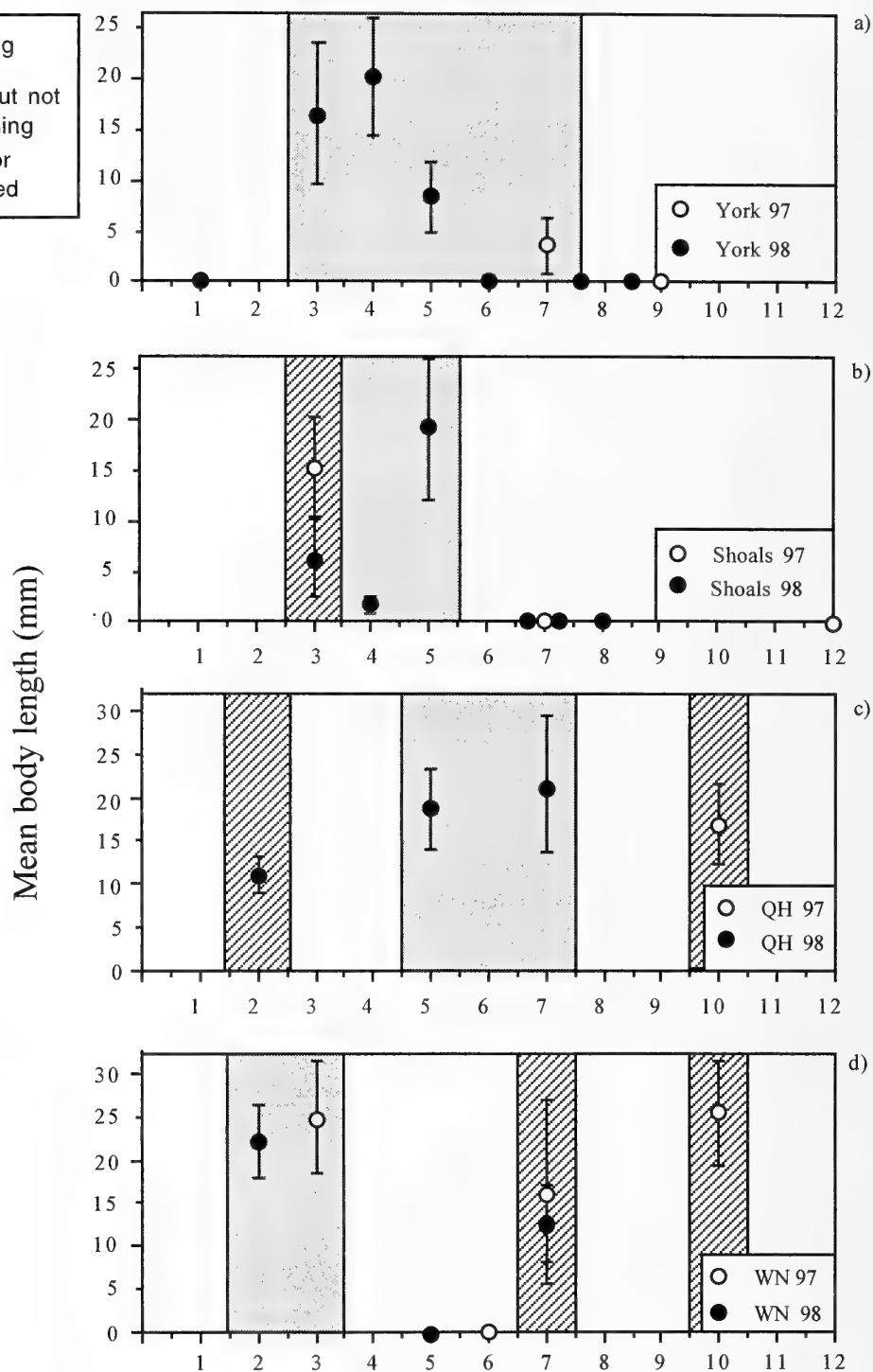


Figure 2. Mean body length ( $\pm$  standard deviation,  $10 < n < 23$ ) of *D. frondosus* versus the month of collection in 1997 and 1998 (January = 1, December = 12). Southern subtidal sites are located at (a) Cape Neddick in York, Maine and (b) the Isles of Shoals, New Hampshire. Northern intertidal sites included (c) West Quoddy Head (QH), Maine, and (d) Wilbur Neck (WN) in Pembroke, Maine. Periods of spawning are shaded between consecutive months.

Table 1

Morphological and developmental characteristics for planktotrophic and lecithotrophic larvae collected in the Gulf of Maine. Included are the zygote diameter upon deposition, the maximum length of the larval shell and the embryonic capsular period from deposition through hatching. Values include means with the number sampled (n)  $\pm$  standard deviation. Zygote diameters ranged between 85–123  $\mu\text{m}$  for planktotrophic larvae and 183–218  $\mu\text{m}$  for lecithotrophic larvae. Feeding capacity was evaluated with micro-algae in laboratory culture conditions (see Methods).

Larval type	Zygote diameter ( $\mu\text{m}$ )	Capsular period (days)	Shell length ( $\mu\text{m}$ )	Feeding capacity
Planktotrophic	102 (70) $\pm$ 12.2	6.7 (11) $\pm$ 2.5	220 (19) $\pm$ 23	Required
Lecithotrophic	194 (70) $\pm$ 14.8	32 (22) $\pm$ 8.9	310 (17) $\pm$ 49	Incapable

subtidal sites would mate with nudibranchs from both of the other two habitats.

### DISCUSSION

There are two disparate life history patterns for *D. frondosus* in the Gulf of Maine. These patterns are most evident at southern subtidal and northern intertidal sites,

while northern subtidal sites may represent overlapping populations between the two types. At southern subtidal sites, the irregular size distributions and timing of spawning events indicate that *D. frondosus* is a fast-growing, more opportunistic predator with a sub-annual seasonal distribution (Todd, 1981). The most common hydroid at these sites, *Obelia geniculata*, is extremely ephemeral and may be nearly exhausted by a combination of predators within only a few months (Lambert, 1991). In contrast, the consistent increase in length among northern intertidal populations of *D. frondosus* followed by a discrete spawning event (Figures 2c, d) indicates that these nudibranchs are relatively slow-growing with an annual seasonal distribution (Todd, 1981). The most common hydroid at these sites, *Sertularia pumila*, is present throughout the year, possibly supplying a constant source of food for these nudibranchs. The senescence of adults after a spawning period (Figures 2c, d) supports the idea of a discrete reproductive event, thus an annual life history pattern (Todd, 1981). The absence of nudibranchs at the Wilbur Neck site following the spawning period (Figure 2d) could be during a period when the larvae are in the water column or when newly recruited individuals are either too small to locate or in a microhabitat different than the adults. These habitats and patterns of feeding ecology have been outlined previously (Meyer, 1971; Clark, 1975; Lambert, 1991; Bleakney, 1996), but not in consideration of the two different larval feeding types produced in these populations and their general biogeographical distribution.

Larvae produced from animals collected at these sites had distinctly different characteristics corresponding with habitat and geographic location. *D. frondosus* produces obligate planktotrophic veliger larvae in southern and northern subtidal habitats. These larvae develop to hatching in a relatively short period of time (Table 1) and may have a longer dispersal potential, corresponding with the opportunistic, highly seasonal occurrence of adult populations. Lecithotrophic veliger larvae are produced by populations in northern intertidal and northern subtidal habitats, and show the potential for limited dispersal by metamorphosing in response to the egg mass jelly. This

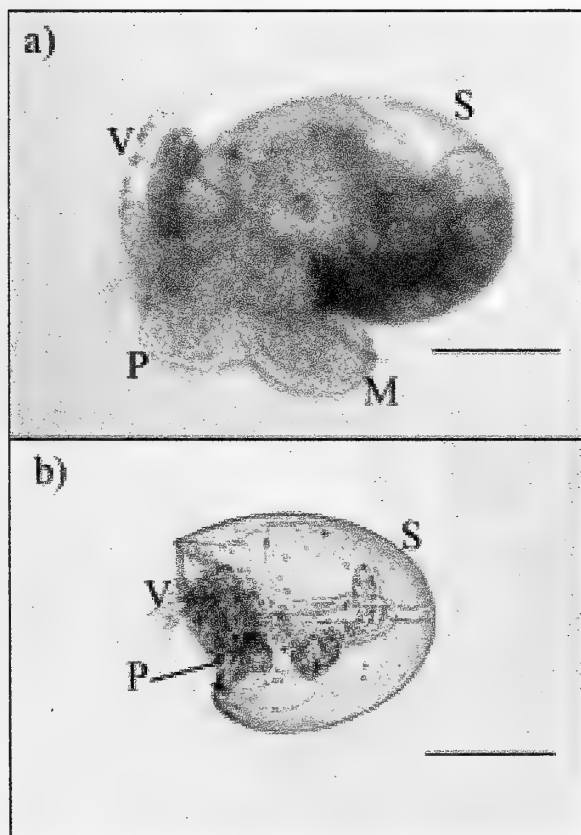


Figure 3. Light micrographs of *D. frondosus* larvae (bar = 100  $\mu\text{m}$ ). a. Lecithotrophic veliger larva from the northern Gulf of Maine. b. Planktotrophic veliger larva from the southern Gulf of Maine. S = shell, V = velum, P = propodium, M = metapodium.

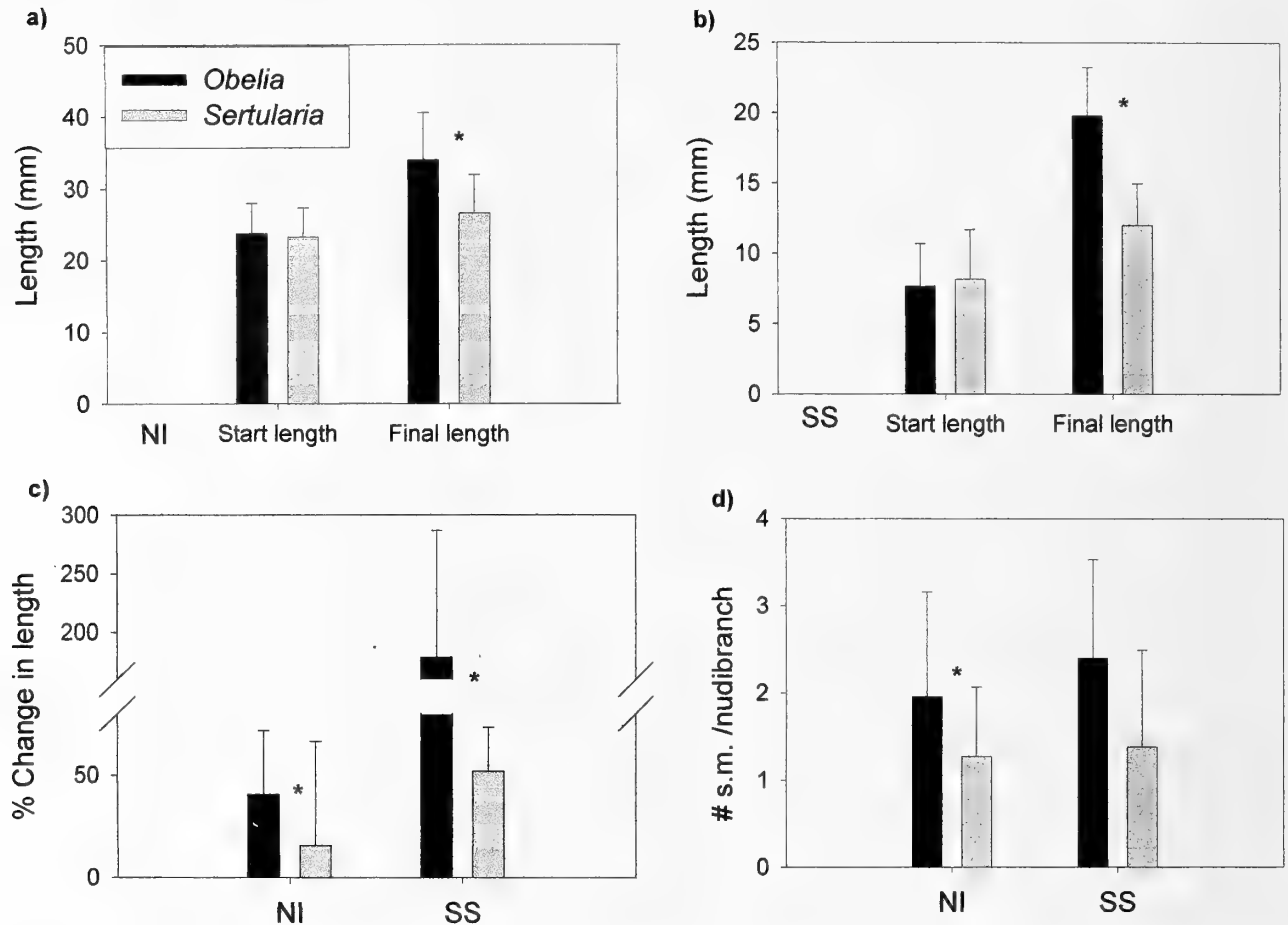


Figure 4. Results from reciprocal feeding experiment with *Obelia* sp. and *Sertularia* sp. fed to *D. frondosus* collected from northern intertidal (NI) and southern subtidal (SS) sites. All results are mean values ( $\pm$  standard deviation) and all comparisons are with a two-tailed t-test, except for the non-parametric Mann-Whitney test for spawn mass products. a. Starting ( $n_{Obelia} = 30$ ,  $n_{Sertularia} = 30$ ,  $t = 0.545$ ,  $df = 58$ ,  $P = 0.59$ ) and finishing ( $n_{Obelia} = 22$ ,  $n_{Sertularia} = 29$ ,  $t = 4.82$ ,  $df = 49$ ,  $P \ll 0.001$ ) lengths for NI trial. b. Starting ( $n_{Obelia} = 25$ ,  $n_{Sertularia} = 24$ ,  $t = 0.533$ ,  $df = 47$ ,  $P = 0.60$ ) and finishing ( $n_{Obelia} = 23$ ,  $n_{Sertularia} = 12$ ,  $t = 6.63$ ,  $df = 33$ ,  $P \ll 0.001$ ) lengths for SS trial. c. Percent change in length (change in length/initial length) for NI ( $t = 3.72$ ,  $df = 49$ ,  $P < 0.001$ ) and SS ( $t = 3.83$ ,  $df = 33$ ,  $P < 0.001$ ) trials. d. Mean number of spawn masses (s.m.)/nudibranch produced by individuals fed the two treatment diets for NI ( $U = 235$ ,  $P \ll 0.001$ ) and SS ( $U = 9.0$ ,  $P > 0.10$ ) trials.

Table 2

Results of mating recognition behavior crosses between *D. frondosus* from northern intertidal (NI) and southern subtidal (SS) sites. Northern intertidal adults always produced lecithotrophic larvae and southern subtidal adults always produced planktotrophic larvae. \* Denotes significant difference ( $G_c = 9.1$ ,  $p < 0.05$ ). † Denotes significant difference ( $G_c = 26.6$ ,  $p < 0.05$ ).

Cross	Copulation observed	No copulation	Total # trials
NI $\times$ NI*	8	10	18
NI $\times$ SS*†	0	18	18
SS $\times$ SS†	15	3	18

response is similar to that found for other opisthobranchs (Gibson & Chia, 1989; Chester, 1996) and although the ability to delay metamorphosis (Pechenik, 1990) may still exist, this limited dispersal potential corresponds with the year-round persistence of adults and may be adaptive for a seasonally constant food source.

Characteristics of egg size and capsular period directly correlate to larval feeding types among nudibranchs (Todd, 1981). Zygote diameters in this study were similar to published values for planktotrophic (Clark, 1975; Strathmann, 1987) and lecithotrophic veligers (Thompson, 1967) for *D. frondosus*. Capsular periods were also in the range of published values for planktotrophic (Hurst, 1967; Williams, 1971) and lecithotrophic (Thompson, 1967) *D. frondosus* veligers. No mixed clutches were

found in any of the habitats, and zygote diameters and capsular periods did not overlap (Table 1).

The dichotomous nature of these characteristics suggests that *D. frondosus* has limited phenotypic plasticity with regard to larval feeding type. In one of the clearest examples of poecilogony, Krug (1998) found mixed clutches of lecithotrophic and planktotrophic larvae in populations of the ascoglossan opisthobranch *Alderia modesta*. Other cases of poecilogony have documented geographically separated populations of a species with different larval types where the adults can interbreed (Levin, 1984; West et al., 1984). The lack of mating recognition between individuals from the populations of *D. frondosus* described in this paper (Table 2) appears to preclude this type of poecilogony.

Quality of adult diet and feeding history has been shown to be an important factor resulting in shifts in larval type for poecilogonous species (Krug, 1998) and changes in capsular period (Gibson & Chia, 1995; Chester, 1996). In this study, reciprocal feeding experiments with adults fed the lower quality diet of *Sertularia* spp. from intertidal habitats and the higher quality *Obelia* spp. from subtidal habitats did not result in any differences in the resulting larval type. Higher growth rates were measured for both types of *D. frondosus* when fed *Obelia* spp., and a high level of mortality was recorded for the *Sertularia* treatment group, especially for the southern subtidal trial. Reduced production of spawn masses on a lower quality diet (*Sertularia* spp.) indicates some gross changes in reproductive effort. Unfortunately, zygote diameters were not measured in these experiments, nor were there any direct measurements of reproductive output in response to these treatments. However, the lack of shift in larval feeding types in response to adult diets of differing quality suggests that this remains a fixed trait for these nudibranch types, and poecilogony is unlikely for *D. frondosus* in the Gulf of Maine. It is important to note here the variation in diameters of zygotes from animals collected in the field despite the consistent disparity between feeding requirements of the resultant larvae (Table 1). Although these life history patterns are dichotomous, there may be considerable variation surrounding each developmental mode. Still, there appears to be little evidence for poecilogony for *D. frondosus* in the Gulf of Maine.

Given the differences in larval type and seasonal patterns of adult occurrence, the separation of populations of this species may not be explained simply by phenotypic plasticity of reproductive traits. This study also evaluated the potential for mating behavior between adults in the northern intertidal and southern subtidal populations. Other mating recognition studies have been used to help clarify sibling species complexes and indicate a strong possibility for reproductive isolation between the two most extreme groups (Hirano & Hirano, 1991; Langan-Cranford & Pearse, 1995). *Dendronotus frondosus*

adults collected from northern intertidal and southern subtidal sites in the Gulf of Maine do not recognize each other as potential mates, representing a distinct reproductive isolation mechanism between these two populations. Only a limited number of replicates from northern subtidal populations were attempted and these yielded similar results by dividing the two reproductive patterns. These data also suggest that the northern subtidal habitat may support sympatric populations of the two types of *D. frondosus* corresponding with the presence of both planktotrophic and lecithotrophic larvae.

The division of life history patterns, larval morphology, and mating recognition behavior is motive for a formal taxonomic review of *D. frondosus* in the Gulf of Maine. This review should include molecular genetic data, comparisons of the radula and reproductive system, and a thorough reanalysis of the literature for the history of this genus in the north Atlantic.

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## A New Species of *Granigyra* Dall, 1889 (Gastropoda: Skeneidae) from Brazil and a Review of Known Western Atlantic Species

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**Abstract.** *Granigyra oblatogyra*, sp. nov. is described based on the shell morphology of seven individuals collected between 510–1250 m off the Brazilian coast. It is characterized by its apically flattened whorls and anteriorly projected aperture. A brief review of the three previously known west Atlantic species is presented. The lectotype of *G. radiata* Dall, 1927, (herein designated), and the holotype of *G. spinulosa* (Bush, 1897) are illustrated by SEM for comparison with the new species.

### INTRODUCTION

The genus *Granigyra* Dall, 1889, comprises a group of minute, trochoid “skeneimorph” gastropods characterized by a sandlike granulation on the shell surface. They are known only from bathyal and abyssal depths, and species are reported primarily from the Atlantic ocean, with a single species from Sumatra (Warén, 1992, 1993). In the western Atlantic, there are species described: *G. radiata* Dall, 1927, from Florida, USA; *G. spinulosa* (Bush, 1897), from the Bahamas; and *G. limata* (Dall, 1889), from Cuba.

The taxonomic position of the genus *Granigyra* is controversial. Hickman & McLean (1990) and Hickman (1998) treated Skeneidae as a polyphyletic assemblage of minute-shelled, non-nacreous taxa of widely differing radular morphology, where many genera might reside provisionally. Warén (1992) adopted a more narrow definition that included a turbinid-like radula and the presence of a unique propodial penis. Although Warén (1992, 1993) found these features lacking in *Granigyra*, he retained the genus provisionally in Skeneidae. In the present study, we follow this provisional classification.

Herein we describe a new species of *Granigyra* collected by dredging carried out during oceanographic expeditions along the Brazilian coast. This is the first record of this genus from Brazilian waters, and review of the other western Atlantic species. In the description section, diameter and number of protoconch whorls were measured following Leal (1991).

Abbreviations used through the text: ANSP—Academy of Natural Sciences of Philadelphia, Philadelphia; IB-UFRJ—Instituto de Biologia/Universidade Federal do Rio de Janeiro, Rio de Janeiro; JOPS—Joint Oceanographic Projects, Research Vessel *Victor Hansen* coll; MNHN—Muséum National d’Histoire Naturelle, Paris; MNRJ—Museu Nacional/Universidade Federal do Rio de Janeiro, Rio de Janeiro; MORG—Museu Oceanográfico

“Eliézer de Carvalho Rios,” Rio Grande; MZSP—Museu de Zoologia da Universidade de São Paulo, São Paulo; PUC—Pontifícia Universidade Católica do Rio de Janeiro, Rio de Janeiro; USNM—National Museum of Natural History, Washington, DC; YPM—Peabody Museum of Natural History/Yale University, New Haven.

### SYSTEMATICS

[?] Family SKENEIDAE Clark, 1851

Genus *Granigyra* Dall, 1889

Type species by monotypy *Cyclostrema*  
(*Granigyra*) *limatum* Dall, 1889

*Granigyra oblatogyra* de Souza & Pimenta,  
sp. nov.

Figures 1–4

**Type material:** Holotype: MNRJ 8433, off Rio Doce, Espírito Santo State (JOPS #3228: 19°45.5'S, 038°45.8'W, 1100 m), length: 2.5 mm, width: 2.4 mm; paratypes: USNM 894863 (JOPS #3228: 19°45.5'S, 038°45.8'W, 1100 m), length: 1.5 mm, width: 1.4 mm; IBUFRJ 11019, off Macaé, Rio de Janeiro State (22°41'25.36"S, 040°26'49.19"W, 780 m), length: 2.3 mm, width: 2.2 mm; MNHN, off Rio Doce, Espírito Santo State (JOPS #3221: 19°50.6'S, 039°34.8'W, 510 m), length: 1.6 mm, width: 1.5 mm (lip broken); ANSP 407933, off Macaé, Rio de Janeiro State (22°37'48.62"S, 043°22'50.14"W, 720 m), length: 2.0 mm, width: 2.0 mm (protoconch broken); MORG 41034, off Macaé, Rio de Janeiro State (22°37'48.62"S, 043°22'50.14"W, 720 m), length: 1.5 mm, width: 1.4 mm; MZSP 32870, off Macaé, Rio de Janeiro State (22°36'52.65"S, 040°40'25.25"W, 1250 m), length: 1.6 mm, width: 1.6 mm.

**Type locality:** off Rio Doce, Espírito Santo State (JOPS #3228: 19°45.5'S, 038°45.8'W, 1100 m), Brazil.

**Range:** Espírito Santo State to north of Rio de Janeiro State, Brazil.

**Diagnosis:** Species with apically flattened whorls and anteriorly projected aperture.

**Description:** Shell medium sized for the genus (holotype: length: 2.5 mm; width: 2.4 mm), turbiform. Surface white, covered with somewhat coarse granulation. Granules increasing in size, following the growth of the shell, resembling striated mountain peaks with flat tops, and much smaller granules in between them (Figure 2). Protoconch (Figure 4) small (diameter: about 220  $\mu$ m), covered with very fine irregular granules, with about one whorl. Teleoconch with 2.5 distinctively rounded, posteriorly flattened whorls that increase rapidly in diameter; connection with previous whorls narrow. Aperture rounded, holostomate, slightly elliptical, projecting slightly anteriorly, with a flat, posterior shoulder next to suture. Umbilicus narrow, slitlike, deep.

**Etymology:** This species is named after its posteriorly flattened whorls (*oblata* = flattened at the poles; *gyra* = turn).

#### *Granigyra limata* (Dall, 1889)

*Cyclostrema* (*Granigyra*) *limatum* Dall, 1889:395 [holotype: USNM 214280, Blake sta. 19, off Bahia Honda, Cuba].  
*Granigyra limata* (Dall, 1889): Dall, 1927:123; Abbott, 1974:56; Warén, 1992: 175, fig. 31E.  
*Lissospira* (*Ganesa* sect. *Granigyra*) *limata* Bush, 1897: 135.

#### *Granigyra spinulosa* (Bush, 1897)

(Figures 5, 6)

*Lissospira* (*Ganesa* sect. *Granigyra*) *spinulosa* Bush, 1897: 135 [holotype: YPM 15805, USFC sta. 2655 (27°22'N, 078°07'30"W)].  
*Granigyra spinulosa* (Bush, 1897): Abbott, 1974:56; Johnson, 1989:65, pl. 10 fig. 1.

#### *Granigyra radiata* Dall, 1927

(Figures 7, 8)

*Granigyra radiata* Dall, 1927:123 [lectotype: USNM 108138, off Fernandina, Florida (herein designated)]; Abbott, 1974:56; Warén 1992: 175, fig. 31E.

**Remarks:** We designate the lectotype of *G. radiata*, as the genus *Granigyra* comprises small somewhat similar

species that may lead to confusing identifications. The species is illustrated for the first time.

**Discussion:** See Warén (1992) for synonymy and taxonomic discussion of the genus.

*Granigyra oblatogyra* has ellipsoid, and posteriorly flattened whorls that distinguish it from *G. limata* (holotype figured in Warén, 1992:232, fig. 31E) that also has much coarser granulation on the body whorl.

The higher shell profile and the ellipsoid, posteriorly flattened whorls of *G. oblatogyra* distinguish it from *G. spinulosa* (Figures 5, 6). In *G. spinulosa* the contact between whorls is wider, and the umbilicus is wider than in *G. oblatogyra*.

*Granigyra oblatogyra* has more convex whorls, a deeper suture, and thinner granulation than *G. radiata* (Figures 7, 8). *Granigyra radiata* also has coarse granules irregularly fused into radially oriented ridges almost parallel to growth lines (Figures 7, 8).

In addition to the western Atlantic species, there are four species in the eastern Atlantic, and one from Sumatra (Warén, 1992, 1993). The most similar to *G. oblatogyra* is *G. granulifera* Warén, 1992:236, figs. 35A–E, but it differs by its nearly to totally disjunct whorls, lower profile, and thinner, less dense granulation.

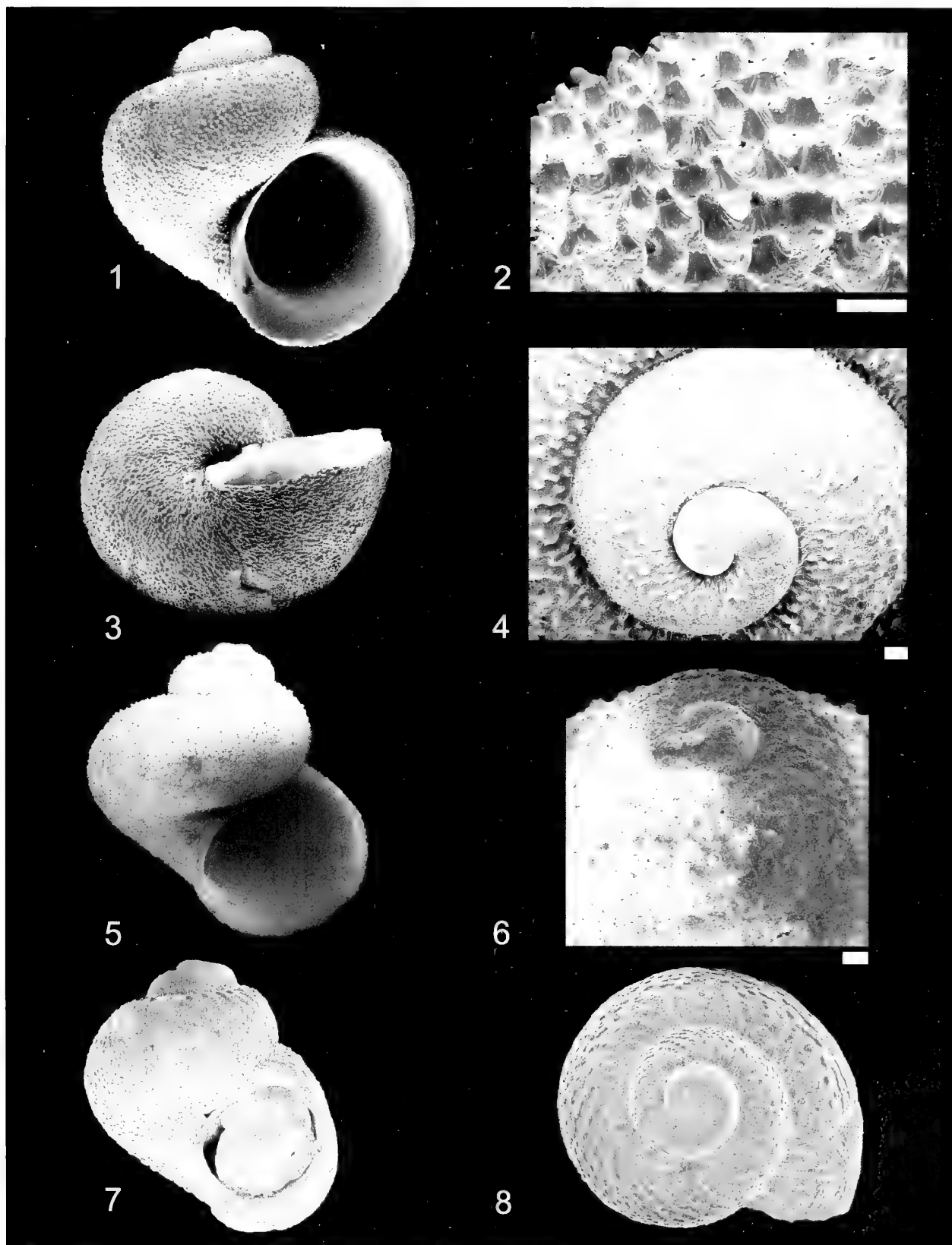
**Acknowledgments.** We are grateful to Dr. E. Lazo-Wasén (YPM) for the loan of the holotype of *G. spinulosa*; Dr. M. Harasewich and Mr. P. Greenhal (USNM) for providing us with the scanning electron micrographs of the lectotype of *G. radiata*; Dr. R. Absalão (IBUFRJ) for providing the specimens of *G. oblatogyra*; Ms. M. F. Lopes (PUC-RJ) and Lara Guimarães (MZSP) for the scanning electron micrographs of the new species; Dr. R. Absalão (IBUFRJ), Dr. C. Hickman, and an anonymous reviewer for their helpful comments on the manuscript. The senior author was funded by doctoral grant FAPESP ("Fundação de Amparo à pesquisa do Estado de São Paulo") 97/11429-3. This study was partially supported by CNPq. ("Conselho Nacional de Desenvolvimento Científico e Tecnológico").

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Figures 1–8. Figures 1–4. Holotype of *Granigyra oblatogyra* de Souza & Pimenta, sp. nov. Figure 1. Apertural view (length: 2.7 mm; width: 2.4 mm). Figure 2. Detail of sculpture, scale 50  $\mu$ m. Figure 3. Ventral view. Figure 4. Detail of protoconch, scale 50  $\mu$ m. Figures 5, 6. Holotype of *Granigyra spinulosa* Bush, 1897. Figure 5. Apertural view (length: 2.3 mm; width: 2.2 mm). Figure 6. Detail of protoconch, scale 50  $\mu$ m. Figures 7, 8. Lectotype of *Granigyra radiata* Dall, 1927. Figure 7. Apertural view (length 2.0 mm; width: 1.9 mm). Figure 8. Dorsal view, showing protoconch and radial sculpture.





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## A New Species of *Attiliosa* (Muricidae: Neogastropoda) from the Upper Eocene/Lower Oligocene Suwannee Limestone of Florida

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**Abstract.** *Attiliosa aenigma*, sp. nov., a muricine muricid, is described from the shallow water, carbonate paleoenvironment of the uppermost Eocene/lowermost Oligocene Suwannee Limestone of Florida. This new species predates all other New World species of *Attiliosa* Emerson, 1969, by roughly 15 ma and is contemporaneous with, or slightly older than, the oldest known fossil species of *Attiliosa* from the Old World. This new occurrence indicates that phylogenetic diversification and geographic range expansion in *Attiliosa* took place much earlier than previously thought. *Attiliosa aenigma*, sp. nov. is most similar in morphology to the Recent *A. bozzettii* Houart, 1993, from Somalia. Both have up to four nodules on the anterior portion of the columella, a posterior channel along the outer lip of the aperture, and fine, closely spaced and paired cords on the upper portion of the body whorl. This latter feature has not been described in muricine muricids until now, although it may have significance for muricine phylogeny.

### INTRODUCTION

In a series of papers revising the systematics and fossil history of the muricid genus *Attiliosa* Emerson, 1968, E. Vokes proposed that *Attiliosa* likely originated in the Old World from within the *Poirieria* clan of the muricid subfamily Muricinae (Vokes, 1971, 1976, 1988, 1989, 1992, 1999; Vokes & D'Attilio, 1982). In support of this hypothesis, Vokes noted potential synapomorphies in the shells of both fossil and Recent *Poirieria* (*Panamurex*) Woodring, 1959, and *Attiliosa*, such as the presence of columellar nodules and labral lirations in the aperture, and general similarities between the radulae of living species of *Attiliosa* and *Poirieria* (Vokes, 1976, 1992, 1999). Vokes' revision of the *Attiliosa* fossil record has also shown that the earliest geological occurrence of the genus is an undescribed species from the early Oligocene of France. The oldest *Attiliosa* in the fossil record of the Americas reported by Vokes is from the late early Miocene Chipola Formation of Florida (Vokes, 1989, 1992, 1999).

In the present study, we describe an enigmatic new species of muricid gastropod from the latest Eocene/earliest Oligocene of Florida, which we refer tentatively to the genus *Attiliosa*. This new fossil species predates all other New World *Attiliosa* by at least 15 ma, and it is roughly contemporaneous with, or possibly even slightly older than, the oldest known species of *Attiliosa* in the

fossil record from France. In addition, we discuss the paleoecology of the Suwannee Limestone in order to provide general information on the ecology and habitats present. Finally, we report a previously undocumented shell character found in certain members of the *Poirieria* clan, which may offer further insight into the phylogeny and evolutionary history of this problematic group.

### GEOLOGY AND PALEOECOLOGY

The most diverse Paleogene molluscan fauna known from Florida (Mansfield, 1937, 1939; Vokes, 1992; Petuch, 1997) occurs at a now disused limestone quarry informally named Terramar 01 (= University of Florida [UF] locality PO017). The quarry is located approximately 9.7 km northwest of Socrum, S 1/4, sec. 10, T. 26 S, R. 22 E, Socrum Quadrangle USGS 7.5' series (1987), Polk County, Florida (Figure 1). Intensive collecting of spoil piles near the water-filled quarry by staff and volunteers of the Florida Museum of Natural History (FLMNH) from 1988 until 1992 yielded numerous silicified invertebrate taxa as well as remains of sirenians and fishes (primarily sharks). During de-watering of the pit in 1990, R.W.P. observed *in situ*, a fine-grained, white limestone underlying an upper silicified zone containing numerous completely or incompletely silicified pseudomorphs of Foraminifera, Cnidaria, Bryozoa, Mollusca, and Echinodermata. Based on lithology and the abundant presence

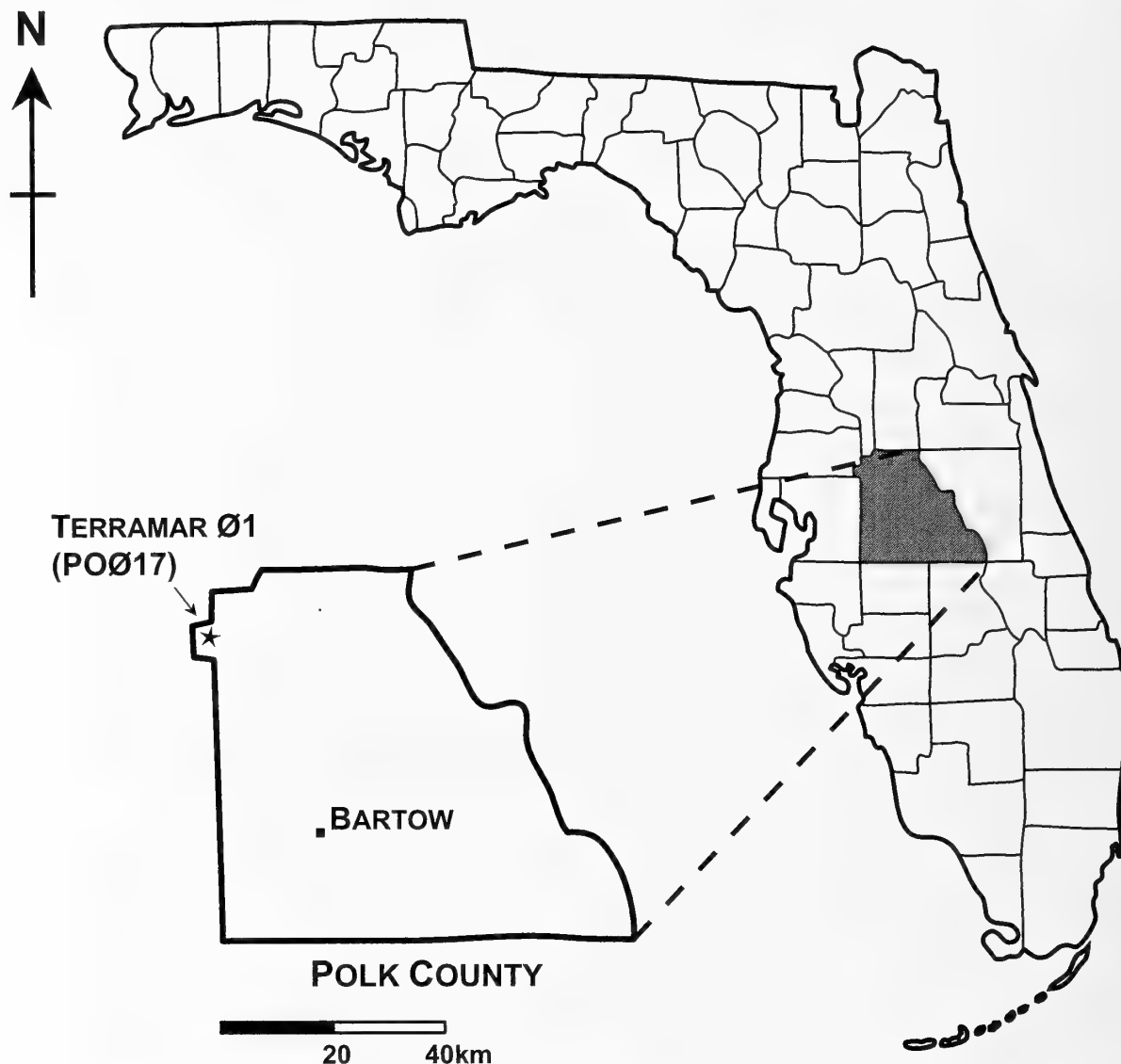


Figure 1. Map of Florida showing location of Terramar 01 (= University of Florida [UF] locality PO017). The quarry is located 9.7 km northwest of Socrum, Polk County, Florida.

of the irregular echinoid *Rhyncholampas gouldii* (Bouvé) throughout the white limestone and silicified zone and the strombid gastropod *Orthaulax hernandoensis* Mansfield, in the silicified zone, the unit was referred to the Suwannee Limestone.

Cooke & Mansfield (1936) originally defined the Suwannee Limestone as a hard, crystalline, yellowish limestone exposed along the Suwannee River near Ellaville, Florida with fossils of *Cassidulus gouldii* (= *Rhyncholampas gouldii*). Typically, the formation is a white to pale orange, soft, and porous wackestone, packstone, or grainstone with loosely cemented foraminifera, common echinoids, and rare to locally abundant mollusks. Moderate variation in lithology exists in the formation

throughout its areal distribution, and induration varies from incompletely cemented to highly cemented to silicified. The Suwannee Limestone is exposed intermittently at the surface from central peninsular Florida to the eastern panhandle region and has been recorded in the subsurface as far south as Key West (Bryan, 1991).

Brewster-Wingard et al. (1997) provided an age estimate for the deposition of the Suwannee Limestone of peninsular Florida using an integrated approach of lithostratigraphic, biostratigraphic (primarily mollusks and dinocysts), and chronostratigraphic (Strontium isotopes) analyses. They determined the Suwannee Limestone to have a depositional age of 36.9 to 30.9 ma ( $\pm 1-3$  ma), which they considered early Oligocene based on the time

scale of Berggren et al. (1985). A revised Cenozoic geochronology presented by Berggren et al. (1995) now places the Eocene/Oligocene boundary at 33.7 ma; thus, deposition of the Suwannee Limestone may have begun during the late Eocene. Although the Brewster-Wingard et al. (1997) study did not analyze Terramar 01 material, Jones et al. (1993) determined an  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope age for the Suwannee Limestone at Terramar to be 33.6 to 34.1 ma ( $\pm 0.5$ –1.0 ma) based on analysis of asteroid (cf. *Goniodiscaster* sp.) marginal ossicles. Following the time scale of Berggren et al. (1995), the Jones et al. (1993) strontium dates indicate the Suwannee Limestone at Terramar 01 straddles the Eocene/Oligocene boundary.

The environment of deposition of the Suwannee Limestone was essentially like that found today off the Florida keys with a shallow water, marine environment floored with carbonate sands and mud and inhabited by a wide range of invertebrates, including corals (Cooke, 1945; Randazzo, 1972; Bryan, 1991; Petuch, 1997). This is generally consistent with what is known of habitat occurrences for modern species of *Attiliosa*, which are commonly collected from 20–30 m depth under coral rubble (Vokes, 1989, 1992, 1999). Several Suwannee Limestone localities contain coral-dominated buildups; and abundant branches of *Stylophora* sp., massive colonies of *Siderastrea* sp., and large heads of *Astrocoenia* sp. have been reported from Terramar 01 (Bryan, 1991). However, based on the common remains of dugongs (sea cows) and the low diversity of branching and massive colonial corals at Terramar 01, the paleoenvironment probably comprised a patch reef and/or coral thickets with sea grass beds, not true reef tracts (Bryan, 1991). Petuch (1997) reported four main substrate types at Terramar 01: bioherms of *Stylophora*; deeper lagoonal open bottom areas; sea grass beds; and very shallow water oyster beds and intertidal mud flats. While Petuch's interpretation of the paleoecology represented by this fauna generally agrees with prior interpretations, it must be pointed out that nearly all the material obtained from Terramar 01 was collected as spoil and that material collected *in situ* during de-watering in 1990 indicated transport. No paired valves of bivalves were found, no preferred orientations were observed, and invertebrate taxa representing different habitats were jumbled together. Clearly, either relatively high wave or current action, as indicated by the presence of small-scale cross beds (Huddleston, 1993), played a role in the formation of this deposit. Furthermore, Petuch's report of an unmapped and still-unstudied Oligocene coral reef tract that developed farther to the west of Terramar 01 is unsubstantiated.

## SYSTEMATIC PALEONTOLOGY

The following locality number and catalogued specimens are those of the Invertebrate Paleontology Division, Florida Museum of Natural History (FLMNH), University of

Florida, Gainesville (collection acronym UF), and the Institut royal des Sciences naturelles de Belgique (IRSNB).

### Class GASTROPODA

### Order NEOGASTROPODA

### Superfamily MURICACEA

### Family MURICIDAE Rafinesque, 1815

### Subfamily MURICINAE Rafinesque, 1815

### Genus ATTILOSA Emerson, 1968

**Type species:** *Coralliophila incompta* Berry, 1960 (= *Peristernia nodulosa* A. Adams, 1855), by original designation.

*Attiliosa aenigma* Herbert & Portell, sp. nov.

(Figures 2a–d)

**Material examined:** Holotype (UF 103371). Height 17.1 mm; maximum diameter 10.3 mm.

**Type locality:** UF locality PO017, Terramar 01 (West Coast Mine), 9.7 km northwest of Socrum, Socrum Quadrangle USGS 7.5' Series (1987), S 1/4, sec. 10, T. 26 S, R. 22 E, Polk County, Florida. Collected from spoil by Roger Portell and Kevin Schindler, November 1989.

**Stratigraphic distribution:** Known only from the type locality.

**Etymology:** *aenigma* (L.) = a mystery or puzzle. A reference to our tentative assignment of the new species to the genus *Attiliosa*.

**Description:** Shell of average size for genus, body whorl inflated. Protoconch and early teleoconch whorls eroded. Spire low, with six visible teleoconch whorls. Spire, last body whorl, and canal (incomplete) each approximately one-third of total shell height. Axial ornamentation comprising nine, thick, rounded ribs on earliest teleoconch whorls, reduced to seven on final whorl. Ribs strong over entire last body whorl, arch-shaped, adherent to previous whorl, and converging with other ribs at suture and tip of siphonal canal. Spiral ornamentation on early whorls not visible due to worm nature of holotype. Final whorl with 15 primary cords of approximately equal strength. Cords paired on adapical portion of penultimate and last whorls. Aperture broad posteriorly, constricted anteriorly. Abapical portion of columella with three or four nodules, the adapical-most nodule being strongest and slightly separated from remaining ones. Low parietal ridge formed by protuberance of rib from previous whorl. Parietal shield broad, adherent to whorl, and flattened ventrally over its abapical half. Adaxial margin of outer lip with eight strong lirae becoming obsolete within. Lirae visible again farther back inside aperture (~ 5 mm from edge of

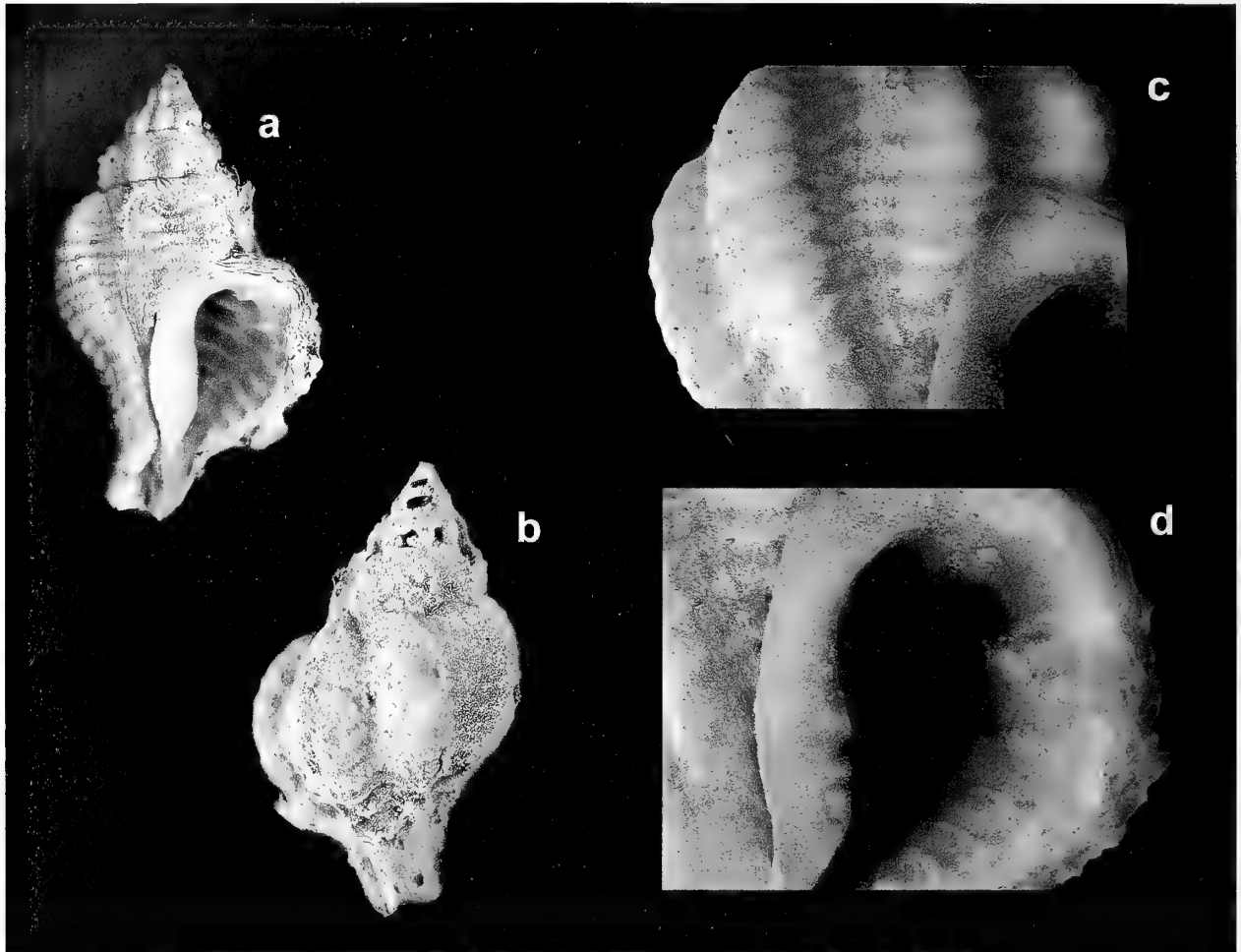


Figure 2. *Attiliosa aenigma* Herbert & Portell, sp. nov. UF 103371 (Holotype); height 17.1 mm, maximum diameter 10.3 mm. Locality: Terramar 01 (PO017), Suwannee Limestone, Polk County, Florida. a. Apertural view. b. Abapertural view. c. View of fine, closely spaced, paired cords on the upper portion of the body whorl. d. Apertural view showing presence of columellar nodules.

aperture) corresponding to resting point at previous lip. Adapical-most lira within aperture separated from anterior seven, delineating a shallow posterior canal. Lower tip and abaxial lip of canal missing. Pre-terminal canals visible over last whorl indicating canal constricted, short, and recurved distally, forming a shallow pseudoumbilicus.

**Discussion:** We assign the new species, *Attiliosa aenigma*, to the Muricinae based on conchological similarities between the holotype and members of the *Poirieria* clan, particularly *Poirieria* (*Panamurex*) Woodring, 1959; *Calotrophon* Hertlein & Strong, 1951; *Dermomurex* (*Takia*) Kuroda, 1953; and *Attiliosa* Emerson, 1968. As in the new species, members of these genera tend to be small (10–30 mm) with inflated body whorls; a broad aperture with a broad parietal shield; lirae on the adaxial margin of the outer apertural lip; six to nine archlike axial elements of equal strength, which extend from the suture to

the tip of the siphonal canal; and an open and slightly recurved siphonal canal.

The combined presence of three additional morphological features of the teleoconch whorls, however, is consistent only with an assignment of the new species to the genus *Attiliosa*. The fine, closely spaced, and paired cords on the upper portion of the body whorl of the new species (Figure 2c), for example, are found in a number of species of *Attiliosa* (Vokes, 1999: figs. 1, 41) and *Takia* (Vokes, 1975: pl. 5, fig. 4; Vokes, 1992: pl. 18, figs. 8, 9) but not *Panamurex* or *Calotrophon* (Vokes, 1992). The presence of columellar nodules in the new species (Figure 2d) is also characteristic of *Attiliosa*, as well as *Panamurex* and *Calotrophon*, but no columellar nodules of this type are found in any species of *Takia* (Vokes, 1992). Lastly, a posterior channel formed along the posterior portion of the aperture in the new species (Figure 2d) is found in several species of *Attiliosa* (Vokes, 1999: figs.

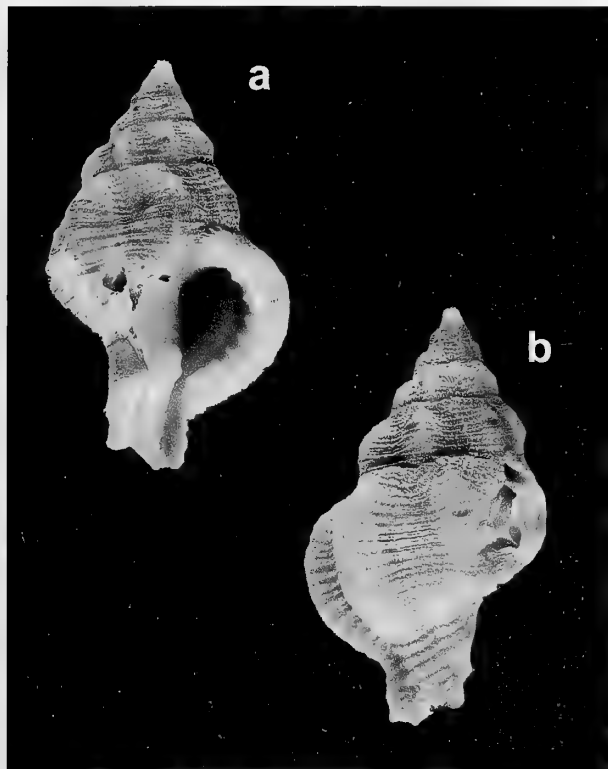


Figure 3. *Attiliosa bozzettii* Houart, 1993. IRSNB IG27.873/454 (Holotype); height 17.0 mm, maximum diameter 10.1 mm. Locality: Ras Hafun, Somalia, 150–200 m. a. Apertural view. b. Abapertural view. (Photographs courtesy of R. Houart)

2, 5, 7) and at least in one species of *Calotrophon* (Vokes, 1992: pl. 19, fig. 11), but no clearly delineated posterior channels are formed in species of *Panamurex* or *Takia*.

Despite this consistency, we regard our generic placement as tentative due to the low number of potentially informative characters in the type material. This problem is attributable, in part, to poor preservation, since the protoconch and early teleoconch whorls are missing in the holotype. More problematic, however, is the relatively simple morphology of the new species, a condition that characterizes a number of sub-lineages within the *Poirieria* clan and has been a prime source of systematic confusion in the Muricinae (Vokes, 1992, 1999). Strengthening our position somewhat is the close morphological resemblance of *A. aenigma* to the living *Attiliosa bozzettii* Houart, 1993 (Figure 3) from deep waters off the coast of Somalia. Both *A. aenigma* and *A. bozzettii* exhibit paired (or bisected) cords, a rounded rather than a shouldered body whorl, and up to four rather than only three columellar nodules, although these characters vary somewhat in *A. bozzettii* (Roland Houart, 2001, personal communication). These species differ in the more pronounced posterior channel in *A. aenigma*.

Similarities to other species of *Attiliosa*, however, even

to the early fossil taxa, are generic only. *Attiliosa aenigma* differs from the undescribed early Oligocene species from France in having a less angulate and sloped body whorl, a broader parietal shield, heavier spiral ornamentation, and a stronger posterior channel. *Attiliosa aenigma* differs from the next earliest New World species, *Attiliosa gretae* Vokes, 1999, of the late early Miocene of Florida, in having a less angulate body whorl, a broader parietal shield, heavier spiral ornamentation, spiral cords of equal rather than unequal strength, a weaker anal channel, and a less recurved siphonal canal.

The new species superficially resembles *Panamurex rutschi* Vokes, 1992 (Vokes, 1992: pl. 11, figs. 1–4) from the Pliocene Punta Gavilán Formation of Venezuela, particularly in the morphology of the axial ribs, apertural lirae, and columellar nodules; however, *P. rutschi* differs in lacking the prominent anal channel and in having thicker, unpaired, and more widely spaced cords on the body whorl. Older species of *Panamurex*, particularly the Paleogene and early Neogene species, all have open spines on the body whorl and siphonal canal, and thus are very different from *A. aenigma*, which lacks spines altogether.

The paired condition of the spiral cords in *A. aenigma* and *A. bozzettii* is noteworthy because the occurrence of similar spiral ornamentation in a number of species of *Takia* (see above), including one of its geologically oldest species, *Dermomurex (Takia) cookei* MacNeil MS in Vokes, 1975, may indicate a closer phylogenetic relationship between *Attiliosa* and *Takia* than previously recognized. Until now, *Attiliosa* has been compared only to *Panamurex* or *Calotrophon* (Vokes, 1971, 1976, 1992, 1999). Detailed studies of the ontogeny of this character and cladistic methods are necessary to determine whether the paired condition is homologous in these different groups. We draw attention to this condition primarily because it has been ignored in past species descriptions and systematic reviews in the literature.

A second fossil muricid (Figure 4) collected from Terramar 01 could be referable to *A. aenigma* because of its nearly identical shell shape, size, and paired spiral cords. However, the axial ribs of this second specimen are narrower, and the columellar nodules are more prominent than in the holotype of *A. aenigma*. Additional material is needed to determine whether this specimen should be included in *A. aenigma* or whether it represents yet another undescribed species.

The discovery of *A. aenigma* has significance for our understanding of the biogeographic history of the genus. Although Vokes (1989, 1992, 1999) proposed that the genus originated in the Old World during the Paleogene and migrated westward in post-Paleogene times, the latest Eocene/earliest Oligocene age of *A. aenigma* and its occurrence in the New World questions this interpretation. Although age resolution of the European material and sampling resolution of the Paleogene fossil record are too



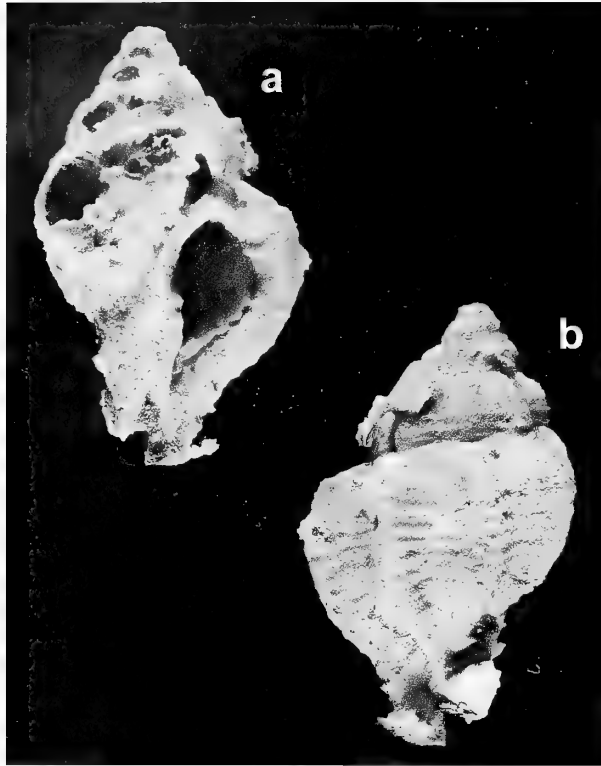


Figure 4. *Attiliosa* sp. cf. *A. aenigma* Herbert & Portell. UF 104450; height 19.0 mm, maximum diameter 11.3 mm. Locality: Terramar 01 (PO017), Suwannee Limestone, Polk County, Florida. a. Apertural view. b. Abapertural view.

poor to determine when and where *Attiliosa* first evolved, the timing and geographic position of the new species indicates, at the very least, that diversification and geographic range expansion in *Attiliosa* were occurring much earlier than previously thought. Future studies should concentrate on refining the systematics of Paleogene Muricidae from the Old World. Are there additional undescribed or "lost" taxa referable to *Attiliosa* and/or closely related groups, and, if so, what do they tell us about character evolution and biogeographic patterns within the Muricinae?

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## Latitudinal Gradients in Body Size and Maturation of *Berryteuthis anonychus* (Cephalopoda: Gonatidae) in the Northeast Pacific

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**Abstract.** Trends in body size and maturation with latitude of the gonatid squid *Berryteuthis anonychus* in the northeast Pacific are described. Squid were collected during May 1999 at seven stations along 145° and 165°W between 39° and 49°N. Mantle lengths ranged from 10.3 to 102.2 mm and increased significantly in both sexes from south to north. Females were both larger and more numerous than males at the northern stations. Both sexes showed a clear pattern of increasing maturity from south to north, and at each station, males were generally in a more advanced stage of maturity than females. Most mature males occurred at the northernmost stations. No mature females were collected. Our data suggest that *B. anonychus* migrates northward in the northeast Pacific during spring, with males maturing at a smaller size than females.

### INTRODUCTION

Most studies of squid migration have been conducted on commercially important species that occur relatively near shore. Some of these species, such as *Todarodes pacificus pacificus* (Steenstrup, 1880), *Dosidicus gigas* (d'Orbigny, 1835), and *Illex illecebrosus* (Lesueur, 1821), have been shown to migrate over long distances (> 1500 km) between low-latitude spawning grounds and high-latitude feeding grounds (Hanlon & Messenger, 1996). Few studies, however, have examined the migration patterns of more oceanic species.

*Berryteuthis anonychus* Percy & Voss, 1963, is a small (mantle length to 150 mm), oceanic squid distributed mainly in the northeast Pacific (Roper et al., 1984). It is a major prey for salmonids (Percy et al., 1988), Pacific pomfret (*Brama japonica* Hilgendorf, 1878; Percy et al., 1993), and neon flying squid (*Ommastrephes bartramii* (Lesueur, 1821); Percy, 1991). Despite its importance in the food web of the subarctic North Pacific, little is known about its life history. In the present study, inferences are made on the migration and spawning of *B. anonychus* in the northeast Pacific based on trends in body size and maturation with latitude.

### MATERIALS AND METHODS

*Berryteuthis anonychus* was collected as by-catch during a United States National Marine Fisheries Service survey of salmon in the northeast Pacific (Carlson et al., 1999). Samples were collected during 6 to 17 May 1999 at seven

stations along 145° and 165°W between 39°01' and 49°03'N (Figure 1). Sampling was conducted from just before dawn to just after dusk. Both longitudinal transects were sampled in different directions (145°W—south to north; 165°W—north to south), and there was no diel pattern to the times at which stations were occupied, thus eliminating the possibility that any trends in body size and maturation with latitude seen in the data might have been due to sampling bias. At each station, a midwater trawl modified to fish at the surface was towed for 1 hour. The trawl was 198 m long with hexagonal mesh in wings and body, and a 1.2 cm mesh liner was used in the codend. Trawling speeds were 7–9 km hr<sup>-1</sup>, and the average net dimensions while fishing were 16 m vertical spread and 45 m horizontal spread. Subsamples of the total catches were taken at the two stations (#11 and #31) where more than 400 squids were collected. A total of 359 *B. anonychus* specimens, including 195 males and 164 females, were examined in the following analyses. Specimens were sexed, and the dorsal mantle length of each was measured. A modified version of the maturity scales described by Lipinski & Underhill (1995) was used for maturity analysis (Table 1). The buccal membrane and inner mantle wall of each female were examined for the presence of discharged spermatophores or spermatangia (sperm vesicles) to determine if any had mated before collection.

The relationship between latitude and mantle length was evaluated using standard regression analysis, and the significance of the population regression was tested using analysis of variance (Zar, 1996:338–343). Normality of the size-frequency distribution at each station was tested using normal quantile plots (Sokal & Rohlf, 1995:118–122). Male and female mantle sizes were compared at

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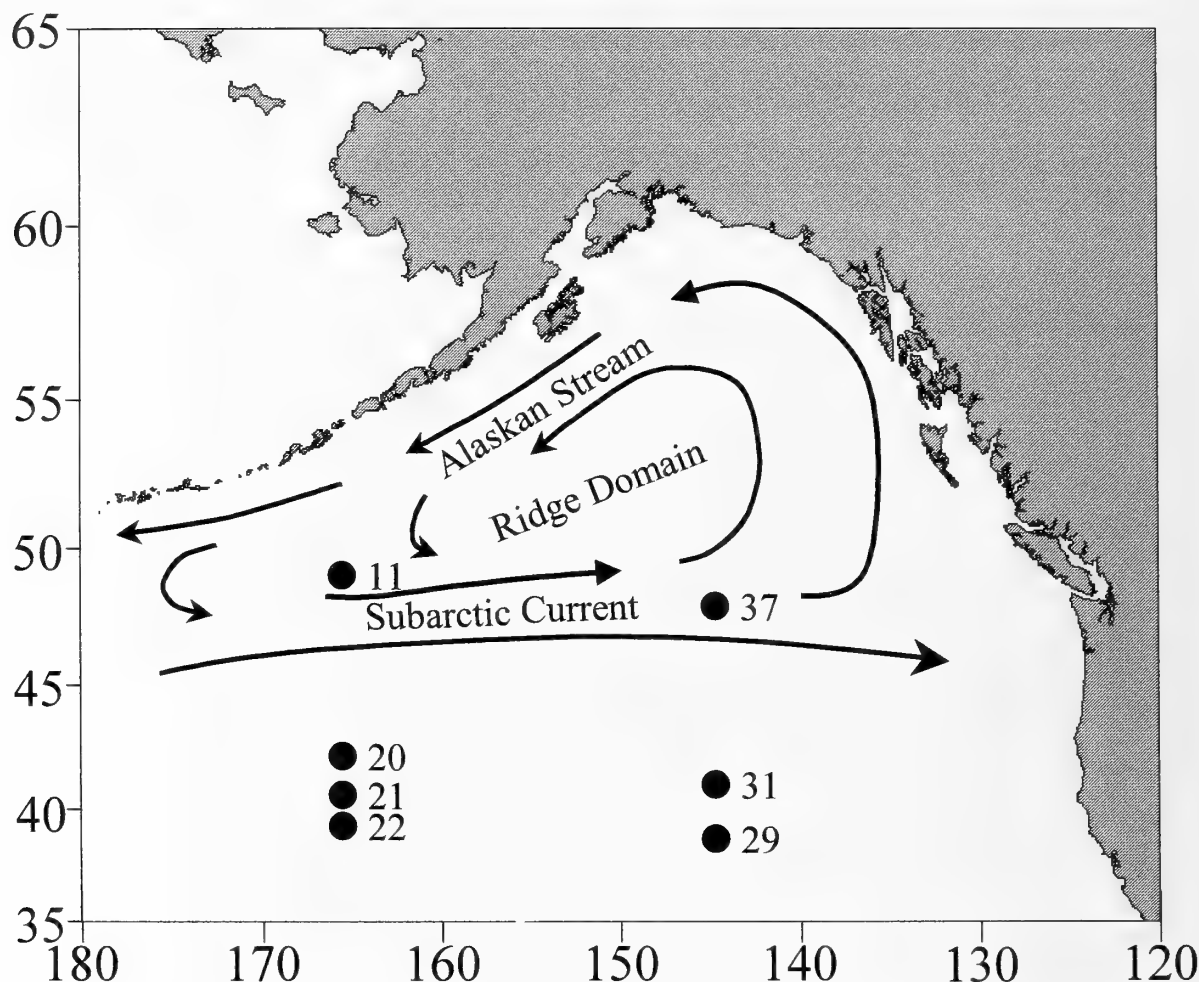


Figure 1. Map of the Northeast Pacific showing sampling stations where *Berryteuthis anonychus* was collected and the long-term mean circulation (adapted from Musgrave et al., 1992). Station numbers correspond to those of Carlson et al. (1999).

each station using the Mann-Whitney test. The relationship between latitude and the proportion of females in the catch was evaluated using Chi-square analysis (Zar, 1996: 562–565); the two southernmost stations, where more than 20% of the samples could not be sexed, were excluded from this analysis. The relationship between maturity stage (dependent variable) and latitude (independent variable) was examined using polytomous logistic regression. Significance in all tests was accepted at the  $P = 0.05$  level.

## RESULTS

### Size

Mantle lengths (ML) ranged from 10.3 to 102.2 mm and increased significantly in both sexes from south to north (Figure 2, Table 2). Station #22 was exceptional in having a wide size range (10.3–75.7 mm ML), including eight specimens larger than 64 mm ML. Size-frequency

data are normally distributed at all other stations except #11, where data are negatively skewed due to differences in size and abundance of males and females.

### Sex

Females were both larger and more numerous than males at the northern stations. At each station north of 42°00'N, male and female sizes differed significantly. This size difference was most distinct at Station 11, where the mean female size was 14 mm larger than that of males. The proportion of females in the catches increased from south to north (Table 2) and followed a significant linear trend. No females had discharged spermatophores or spermatangia present in the buccal membrane or inner mantle wall, suggesting that none had mated before collection.

Table 1

Maturity scale used for *Berryteuthis anonychus* (adapted from Lipinski & Underhill 1995). ML, mantle length; NG, nidamental gland; NGI, nidamental gland index = NG length/MLX100; NS, Needhams' sac; OG, oviducal gland; OM, oviducal meander; PL, penis length (distance from the anteriormost part of the spermatophoric complex to the distal (anterior) end of the penis); PLI, penis length index = PL/MLX100; S, spermatophores; SC, spermatophoric complex.

Stage	Male stage description					Female stage description				
	Testis	Separate parts of SC visible	PLI	S in NS	S in penis	Mantle thin and/or flaccid	Ovary	Nidamental glands	Oviducts	Ovaries contain mature oocytes
I Juvenile	transparent	no	< 5	0	0	no	translucent	small and transparent; NGI < 6	not visible	no
II Immature	whitish; structure not visible	yes	< 5	0	0	no	opaque	transparent or opaque; NGI < 6	not visible	no
III Preparatory	whitish; structure not visible	yes	5-15	0	0	no	opaque	enlarged and opaque; NGI > 6	visible; contain no eggs	no
IV Maturing	whitish; structure visible	yes	> 15	≤ 2	0	no	opaque	opaque; NGI > 6	visible; OM contain a few eggs	a few
V Mature	whitish; structure visible	yes	> 15	densely packed	present	no	opaque	opaque; NGI > 6	visible; OM contain many eggs	many
VI Spent	whitish; structure visible	yes	> 15	few	present	yes	opaque	opaque; NG > 6	many eggs visible; few eggs remain	yes

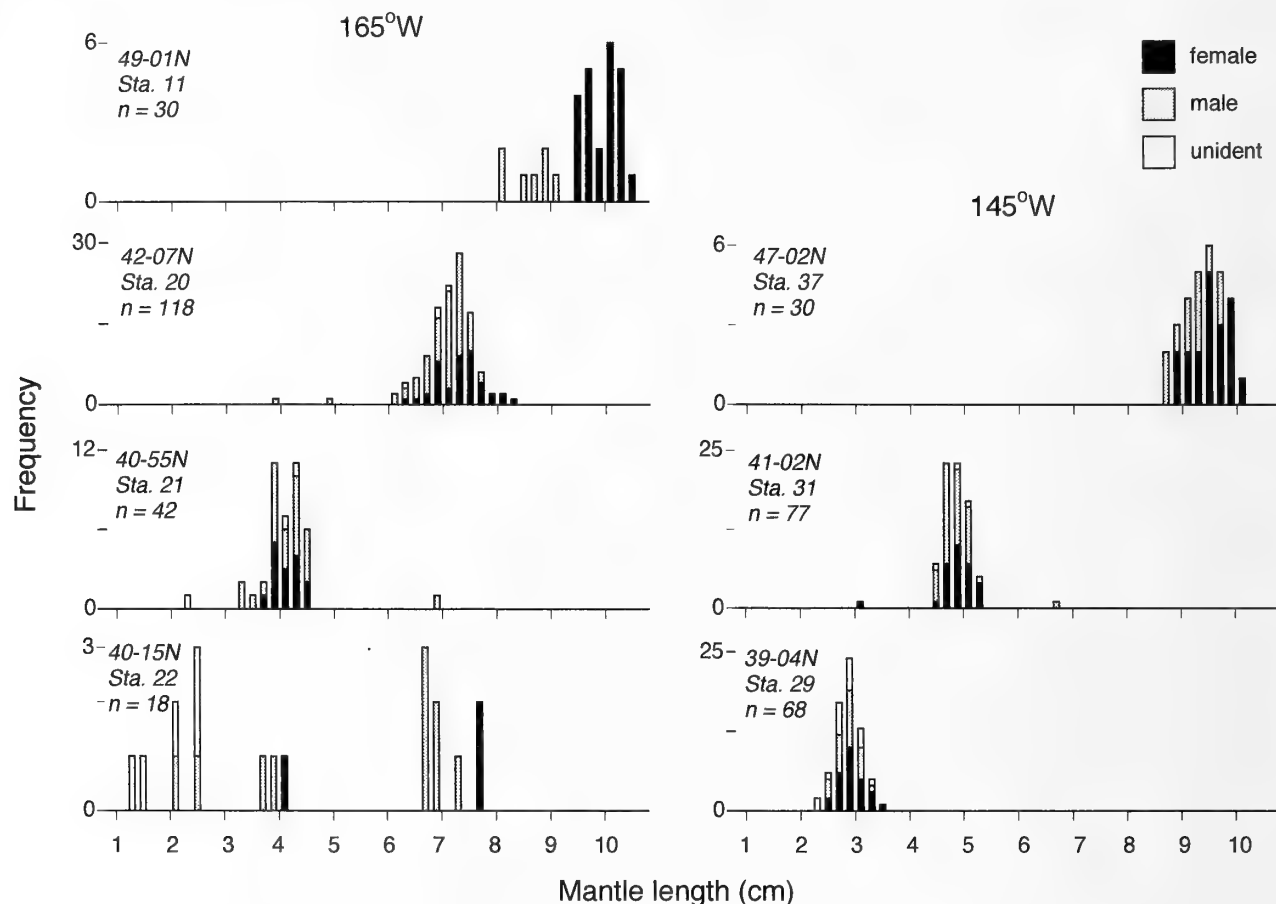


Figure 2. Frequency distributions for mantle size of *Berryteuthis anonychus* collected in the northeast Pacific along 145° and 165°W.

### Maturity

Both sexes showed a clear pattern of increasing maturity from south to north (Figure 3), and the relationship between maturity stage and latitude was significant. At each station, males were generally in a more advanced maturity stage than females. Mature (stage V) males occurred at four stations between 40°15' and 49°01'N and

ranged in size from 64.1 to 94.8 mm ML; 71% were collected at the two northernmost stations. The seven mature males collected at and south of 41°02'N differed significantly in size from those at the two northernmost stations (Mann-Whitney test,  $P < 0.001$ ). Of males larger than 75.8 mm ML, 94% were mature. Females ranged in size from 23.3 to 102.2 mm ML, but none were mature. Of the most advanced female maturity stage collected (stage III), 89% were collected at the two northernmost stations. The five stage III females collected at and south of 42°07'N differed significantly in size from those at the two northernmost stations (Mann-Whitney test,  $P < 0.001$ ). Station #22 was again exceptional in being the only southern station where advanced maturity stages of both sexes were collected.

### DISCUSSION

*Berryteuthis anonychus* collected during spring in the northeast Pacific increased in size and maturity from south to north. Didenko (1990, in an abstract from the 5th All-USSR Conference on Commercial Invertebrates in 1990) reported similar trends in size and maturity with

Table 2

Median mantle length (ML) and % of females in the catch of *Berryteuthis anonychus* at each station.

Latitude (N)	Longitude (W)	Station #	Median ML (mm)	Female %
49°01'	165°	11	95.7	77
47°02'	145°	37	92.2	63
42°07'	165°	20	69.6	38
41°02'	145°	31	46.5	42
40°55'	165°	21	39.3	39
40°15'	165°	22	38.1	
39°04'	145°	29	26.8	

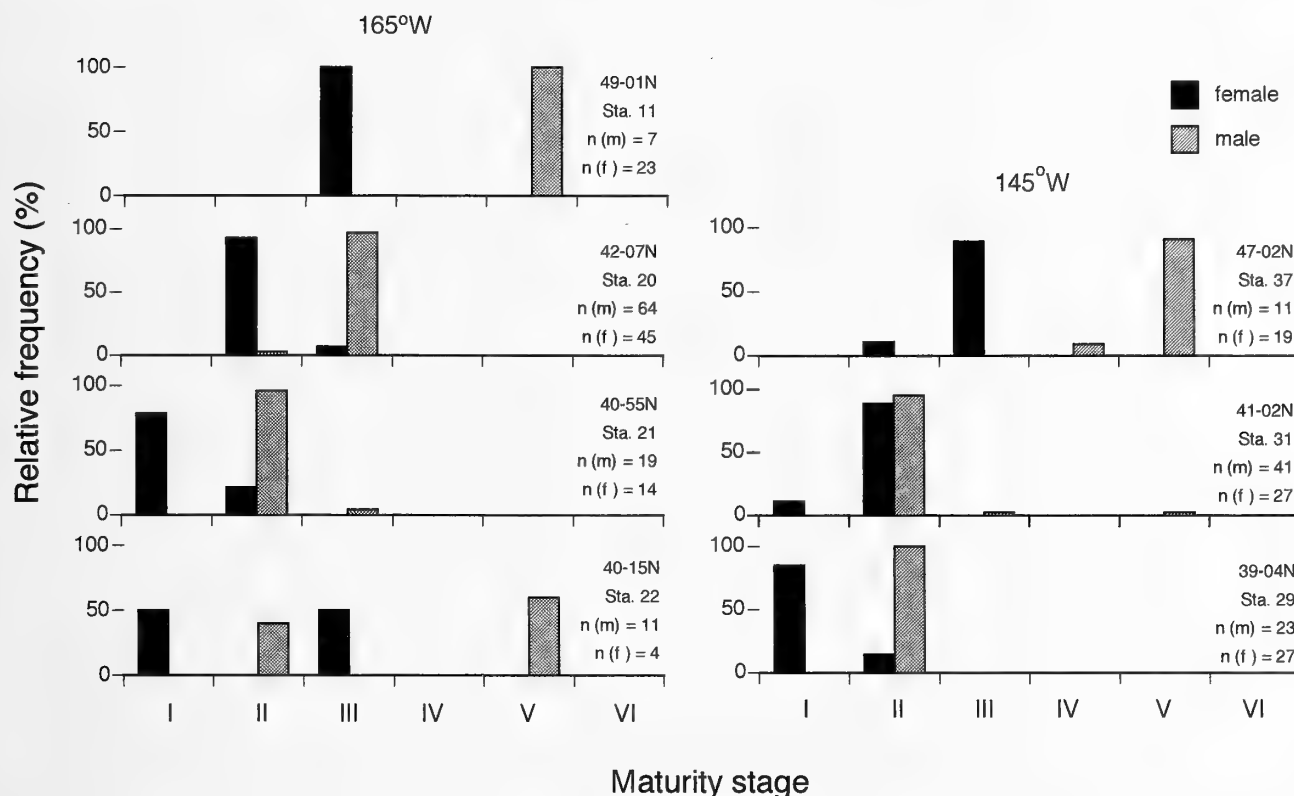


Figure 3. Relative frequency distributions for maturity stage of *Berryteuthis anonychus* collected in the northeast Pacific along 145° and 165°W.

latitude in this area during spring and summer. In the absence of known northward currents in this area, the simplest explanation for these patterns is that *B. anonychus* actively migrates northward during spring and summer.

*Ommastrephes bartramii*, another pelagic squid distributed widely in the North Pacific (Clarke, 1966), shows a similar trend of increasing size with latitude as it migrates northward during summer and fall. It hatches in subtropical waters, migrates to feeding grounds north of 41°N, then returns south of 32°N to spawn (Murata & Nakamura, 1998). As it migrates northward along 165°W, modal mantle lengths increase from 15 cm near 37°N to 40 cm near 46°N (Murata & Hayase, 1993), which is a mean increase in mantle length of about 19% per degree latitude. *Berryteuthis anonychus* modal mantle lengths in the present study increased from about 3 cm near 39°N to about 10 cm near 49°N, for a similar mean increase in mantle length of about 23% per degree latitude.

The sizes of *Berryteuthis anonychus* found in predator stomachs collected in the Subarctic Current in the northeast Pacific during summer roughly correspond with the relation between body size and latitude seen in the present data. Pacific pomfret (*Brama japonica*) at 49–52°N prey heavily on > 70–80 mm ML squid (Pearcy et al., 1993),

and salmonids at 45°30'–51°N prey heavily on 80–100 mm ML squid (Pearcy et al., 1988). In the Ridge Domain and Alaska Stream north of the Subarctic Current, small (< 60 mm ML) *Gonatus* spp. squids replace the larger *B. anonychus* as the main cephalopod prey of both Pacific pomfret and salmonids (Pearcy et al., 1988; Pearcy et al., 1993), suggesting that it becomes more difficult to prey on *B. anonychus* as it increases in size.

Males matured at a smaller size than females. These data are consistent with those of other gonatids, including *Berryteuthis magister magister* (Berry, 1913), *Gonatopsis borealis* Sasaki, 1923, and *Gonatus onyx* Young, 1972 (Arkhipkin et al., 1996; Nesis, 1997). The occurrence of small maturing specimens south of 41°N suggests that early maturing forms may occur in southern waters. Early- and late-maturing groups have been reported in other pelagic squids, including *G. borealis*, and the ommastrephids *Sthenoteuthis pteropus* (Steenstrup, 1855), *S. oualaniensis* (Lesson, 1830), and *Dosidicus gigas* (Nesis, 1997; Masuda et al., 1998).

Both sexes were of similar size and abundance at southern stations, but females became larger and more numerous than males at the northern stations. Differential growth of the sexes is common in cephalopods as they approach maturity (Forsythe & Van Heukelem, 1987),

with females growing larger than males in many oceanic squids, including *Ommastrephes bartramii* (Yatsu et al., 1998) and *Berryteuthis magister magister* (Natsukari et al., 1993). Off northeastern Japan, the sex ratio in catches of *O. bartramii* at the northern feeding grounds (42–44°N) is nearly even, but the proportion of females increases as they approach the southern spawning grounds (Murata & Ishii, 1977).

The spawning habitat of *Berryteuthis anonychus* is unknown, but two possible spawning scenarios can be surmised based on the present results. The first is that, like *Ommastrephes bartramii*, after feeding and growing in northern waters, *B. anonychus* returns to spawn south of 39°N, where the smallest specimens were collected in the present study. Such a pattern is consistent with the “one-return journey” migration pattern between low-latitude spawning grounds and high-latitude feeding grounds commonly seen in migrating pelagic squids, such as *Dosidicus gigas* (Nesis, 1983), *O. bartramii* (Murata & Nakamura, 1998), and *Todarodes pacificus pacificus* (Okutani, 1983)). A second, and more intriguing, scenario is that the main spawning grounds occur in northern waters. *Berryteuthis anonychus* paralarvae (ML < 10 mm) occur during summer in and near the Alaska Stream (Kubodera & Jefferts, 1984; J. R. Bower, unpublished data), indicating that hatching indeed occurs in the northern Gulf of Alaska. *Berryteuthis anonychus* may spawn near the seafloor along the continental slope as its congener *B. magister magister* does (Nesis, 1997).

A northern-spawning-ground scenario would require southward currents to transport egg masses and paralarvae to at least 39°N, where the smallest specimens were collected in the present study. Planktonic larvae of *Enteroteuthis dofleini* (Wülker, 1910) that hatch in coastal waters along the Aleutian Islands occur along 180° longitude as far south as 45°N, 700 km south of the islands (Kubodera, 1991). Darnitsky et al. (1984) described the southerly movement of water from the Alaskan Stream along 170°E as far south as 40°N and suggested that it plays a role in transport of plankton from northern waters to the southern Emperor-Northern Hawaiian Ridge seamounts. These observations suggest that there are currents in this area that could transport *Berryteuthis anonychus* eggs and paralarvae spawned near the Aleutian Islands southward.

Clearly more data are needed, particularly collected over wide geographical and temporal scales, before definitive conclusions can be drawn on the complete migratory behavior of *Berryteuthis anonychus* in the northeast Pacific. The present study provides the first step in trying to understand the life history of this little studied, yet ecologically important, squid.

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## Ultrastructure of Muscle-Shell Attachment in *Nautilus pompilius* Linnaeus (Mollusca: Cephalopoda)

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**Abstract.** The ultrastructure of the muscle-shell attachment in the embryo and adult specimens of *Nautilus pompilius* Linnaeus, 1758, was investigated by optical and transmission electron microscopy. In adult specimens, myo adhesive mantle epithelial cells at the attachment site of the retractor muscle are high columnar and characterized by elongate microvilli having undulate cytoplasmic membranes, numerous bundles of fibrils, and interconnection with neighboring cells by means of interdigitation. The inner shell wall of the body chamber at the attachment site is covered by a thick (approx. 80  $\mu\text{m}$  thick in adult animals) semi-transparent membrane. The tips of the microvilli are very thin and intertwined with each other, and do not insert into the inner surface of the semi-transparent membranes. Similar features are also observed in the myo adhesive cells at both the attachment site of the retractor muscle and the initial portion of the siphuncular cord of the embryo.

The myo adhesive epithelium-semi-transparent membrane junction of *N. pompilius* seems to be physically weak against tensile stress caused by muscle movement. The peculiar mode of muscle-shell attachment in *Nautilus* appears to have developed as a result of adaptation to a nektonic mode of life and mode of shell growth followed by a chamber formation cycle.

### INTRODUCTION

Calcareous hard exoskeletons of mollusks play important roles in protecting soft parts from ambient environments and providing a solid base for muscle attachment. In all mollusks hitherto examined histologically, a collagenous intercellular matrix and specialized epithelial cells (myo adhesive cells) intervene between the muscle fibers and the shell (e.g., Hubendick, 1958; Nakahara & Bevelander, 1970; Tompa & Watabe, 1976). On the inner shell surface, the attached area of the myo adhesive cells is distinguished with a unique shell structure from the non-attached area by the presence of variably depressed scars, which correspond to the exposed surface of the myostracum. Such attachment scars provide a reliable key to reconstructing the muscle system of fossil mollusks. Therefore, many biologists and paleontologists have focused on the attachment scars in molluscan shells from the viewpoints of taxonomy, functional morphology, and physiology. Ultrastructural features of the muscle-shell

attachment may also provide an important information source to improve our understanding of the paleobiology of extinct mollusks.

Ultrastructural features of the muscle-shell attachment have been investigated in bivalves (Nakahara & Bevelander, 1970), gastropods (Tompa & Watabe, 1976), monoplacophorans (Haszprunar & Schaefer, 1997), and scaphopods (Shimek & Steiner, 1997). As a result of these works, it was realized that the myo adhesive cells are differentiated into cuboidal, fiber-rich cells having short microvilli, which are basically common among different taxa.

*Nautilus* is the sole living genus of the ectocochleate cephalopods. Various attachment scars impressed on the inner shell surface are the only direct evidence of the shape and location of the attachment of the soft body to the shell. Previous authors have focused mainly on the shape and location of the attachment scars in the shell of *Nautilus* (e.g., Grégoire, 1962; Tanabe et al., 1991; Mut-

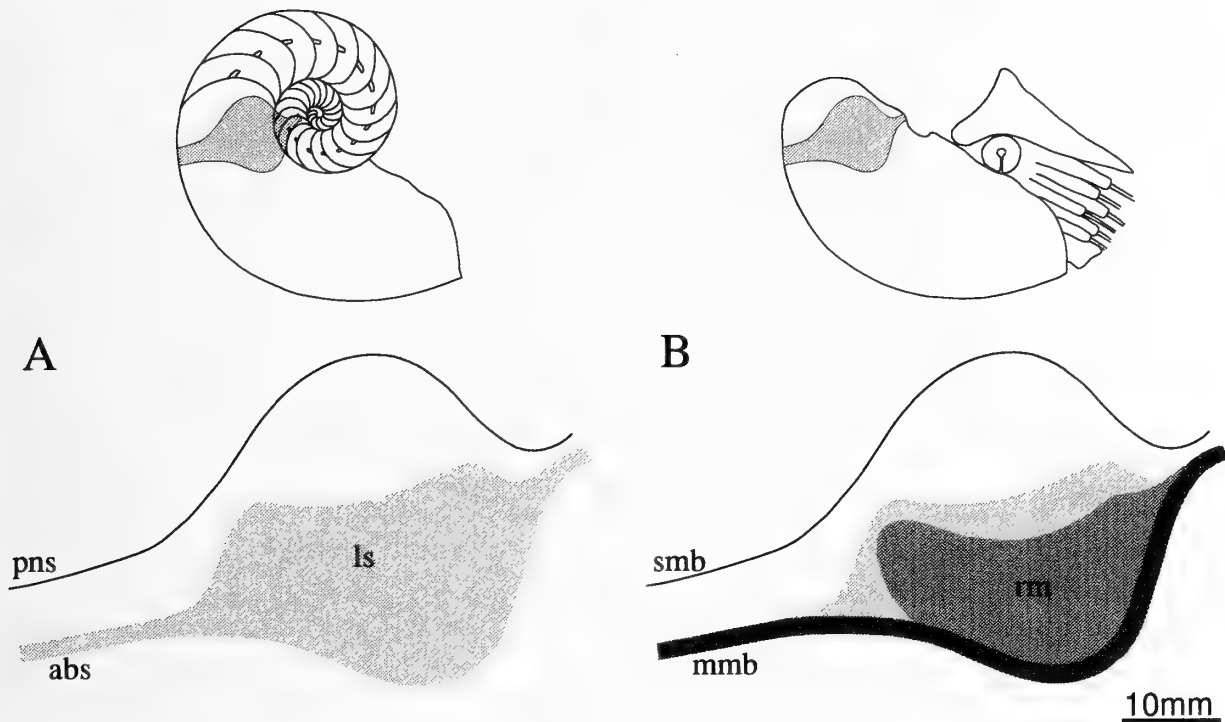


Figure 1. Shape of the attachment scar and the corresponding muscle termination in *Nautilus* (lateral views). A. Attachment scar on the left side of the body chamber. B. Mirror image of the muscle termination of the left side of the body. Key: abs, anterior band scar; mmb, mantle myoadhesive band; ls, large scar; pns, posterior narrow scar; rm, retractor muscle; smb, septal myoadhesive band.

vei et al., 1993; Doguzhaeva & Mutvei, 1996; Mutvei & Doguzhaeva, 1997), but the details of the muscle-shell attachment have been little investigated except for light microscopic observations carried out by Bandel & Spaeth (1983). Bandel & Spaeth (1983) reported that the high columnar myoadhesive epithelial cells have elongate microvilli, whose structure differs from those in the other molluscan groups. Such differences may have specific functional significance; yet no observations by means of transmission electron microscopy have been done on the muscle-shell attachment.

The purpose of this paper is to describe the ultrastructural features of muscle-shell attachment in *Nautilus pompilius*. It also discusses possible functional explanations of the attachment system of *Nautilus* in relation to the animals' nektonic mode of life and their mode of growth involving the chamber formation cycle.

#### MATERIALS AND METHODS

One adult (approx. 200 mm diameter) and one young adult (135 mm diameter) of *Nautilus pompilius* Linnaeus,

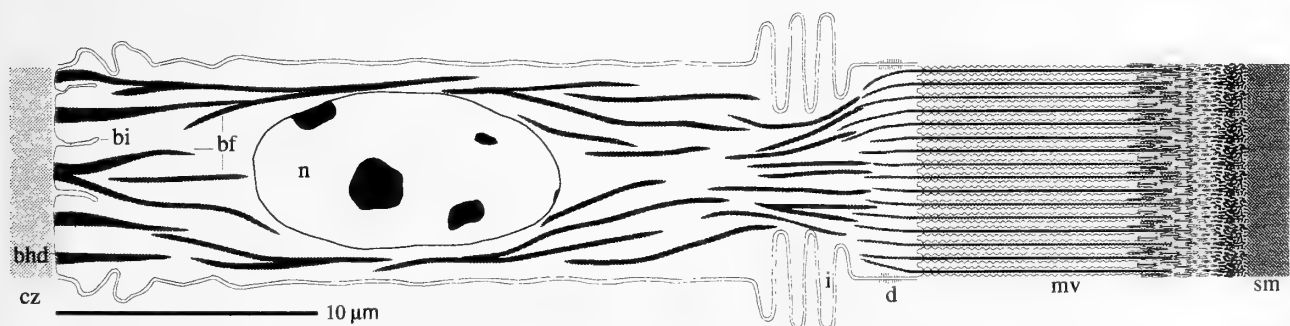


Figure 2. Diagrammatic representation of a myoadhesive epithelial cell located at the attachment site of the retractor muscle of the adult animal of *Nautilus pompilius*. Key: bf, bundles of fibrils; bhd, basal hemidesmosome; bi, basal infolding; cz, collagenous zone; d, desmosome; i, interdigitation; mv, microvilli; n, nucleus; sm, semi-transparent membrane.

1758, were collected off Tagnan, Panglao Island, Bohol, the Philippines, on 14 May 1996. One embryo came from an egg laid on 6 June 1997, at the Toba Aquarium by an adult animal, which was captured from off Taar area, southern Luzon Island, the Philippines. The embryo was taken from the egg capsule on 6 November 1997, after incubating 154 days at a mean temperature of 23°C.

For observation of the shapes of attachment scars and the corresponding lateral termination of the attachment muscle, one young adult animal was examined. The animal was fixed with 10% formalin without decalcification. Subsequently, the shell was cut along the medial axis, and the soft tissue was carefully removed from the shell for observation under a binocular microscope.

One adult and the embryo were examined by transmission electron microscopy (TEM). In the case of the adult specimen, the shell was carefully removed from the soft tissue without decalcification, and the mantle epithelium at the attachment site of the retractor muscle to the inner shell wall was sectioned into small pieces and fixed with 2% paraformaldehyde/2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.5) for several days. The preparation of the embryo was done in the following manner. After removing the yolk mass, the whole body of the embryo was fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.5) with 6% sucrose added for osmolarity for several days. It was subsequently decalcified with 4.13% EDTA buffered to pH 7.5, and the epithelial portion was sectioned into two pieces along the ventrodorsal plane, one at the attachment site of the retractor muscle and the other at the mid-apical portion which attached to the inner shell wall. Pieces of soft tissue of the adult and embryonic specimens were subsequently washed in cacodylate buffer for 4 hours and post-fixed in 2% osmium tetroxide for 1.5 hours. After dehydration in an ethanol series, they were embedded in Epon 812 resin for the adult materials and in Spurr resin for the embryonic ones. Ultra-thin sections were prepared for the tissue materials with a diamond knife using an LKB-Ultratome. Sections of the adult specimens were stained with uranyl acetate and lead citrate. Those of the embryo specimens were stained with potassium permanganate and lead citrate to emphasize the contrast of electron density. These sections were examined and photographed with a Jeol JEM-1200EX II TEM. One- $\mu$ m-thick sections were stained with toluidine blue for optical microscopy.

Terminology used in this description is partly that used by Mutvei & Doguzhaeva (1997).

## RESULTS

### Shape of Attachment Scar and Muscle Termination

The annular attachment scars occur on the lateral and dorsolateral sides of the inner wall of the body chamber in front of the last septum. Figure 1A shows the lateral view of one half of the annular attachment scars preserved on the left inner side of the body chamber. The scar is divisible into anterior and posterior parts. The anterior portion of the scar comprises an anterior band scar and a large scar (Figure 1A; abs + 1s). The large scar is somewhat trapezoidal in shape showing anterior round and posterior slightly angular outlines. The posterior scar, in contrast, is expressed as a sharply impressed, very narrow annular scar (Figure 1A; pns). The annular myoadhesive epithelial regions that produce these scars encircle the body. Figure 1B shows the mirror image of the myoadhesive epithelial regions on the left side of the body. These regions compose the annular band of origin of the longitudinal mantle muscles (= mantle myoadhesive band: mmb), large lateral termination of the retractor muscle (rm), and a narrow annular band, along which the muscles from the septal portion of the body wall take their origin (= septal myoadhesive band: smb) in order from anterior to posterior side of the body. The attachment area of the retractor muscle is crescent-shaped and overlaps the mantle myoadhesive band at the anterior edge.

The septal myoadhesive band (smb) is equal in shape to the corresponding attachment scars (pns). The anterior edge of the mantle myoadhesive band (mmb) also corresponds to the anterior line of the anterior attachment scar (abs). In contrast, the posterior edge of the large scar (1s) is broader than the lateral termination of the retractor muscle (rm). This fact indicates that the myoadhesive epithelial area is broader than the lateral termination of the attachment muscle.

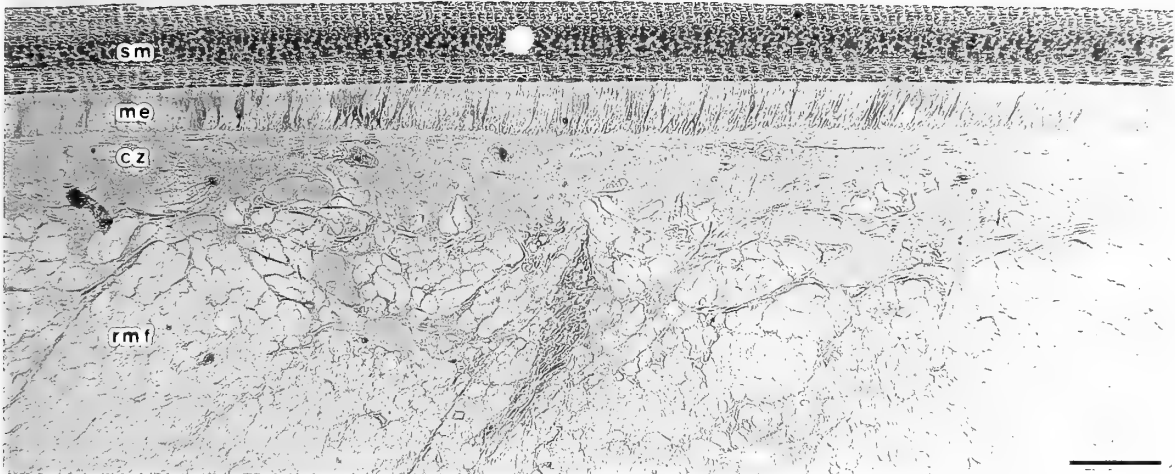
### Ultrastructure of Muscle-Shell Attachment

In *Nautilus*, the muscle fibers terminate in a collagenous zone at the base of the myoadhesive epithelium.

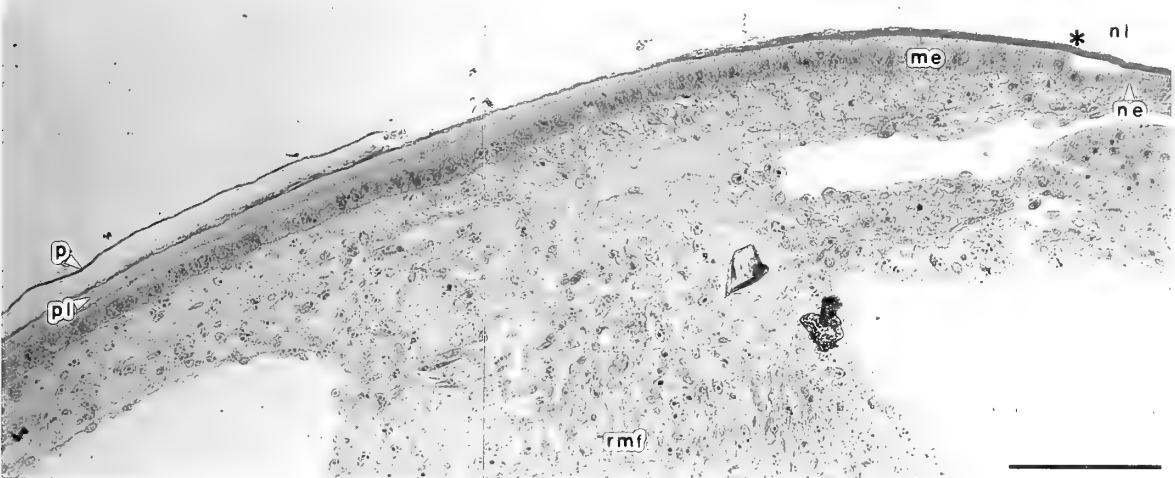
Figure 3. Light micrographs of the longitudinal sections of the myoadhesive epithelial regions of adult and embryonic animals of *Nautilus pompilius*. A, Retractor muscle attachment of the adult. B, Retractor muscle attachment of the embryo. Enlarged view at the ventral edge of attachment site (asterisk) is shown in Figure 7. C, Initial portion of the siphuncular cord of the embryo. Enlarged view at the ventral edge of attachment site (asterisk) is shown in Figure 9. Key: cz, collagenous zone; me, myoadhesive epithelium; rmf, retractor muscle fiber; ne, non-adhesive epithelium; nl, nacreous layer; p, periostracum; pl, prismatic layer; sm, semi-transparent membrane. Arrows indicate ventral direction. Scale bar = 100  $\mu$ m.

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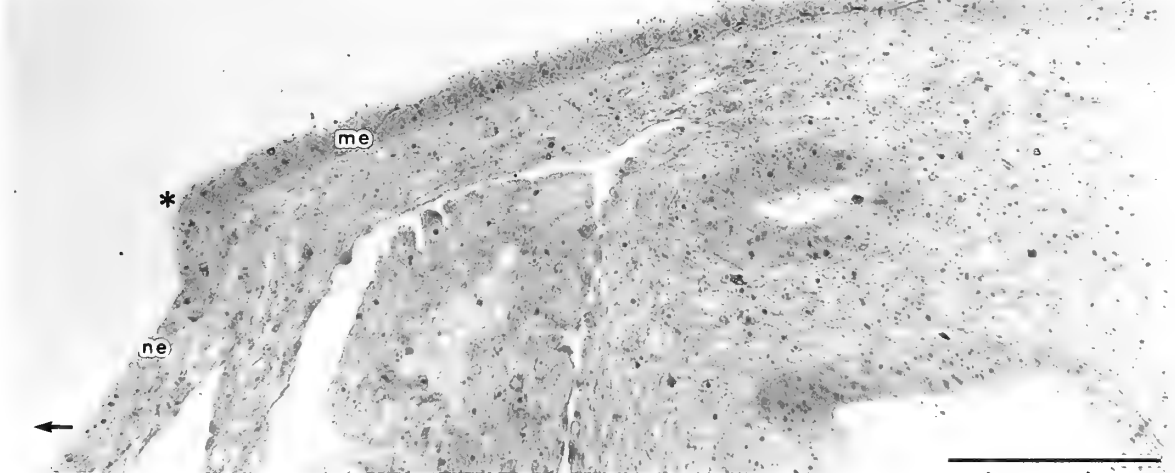
**A**



**B**



**C**



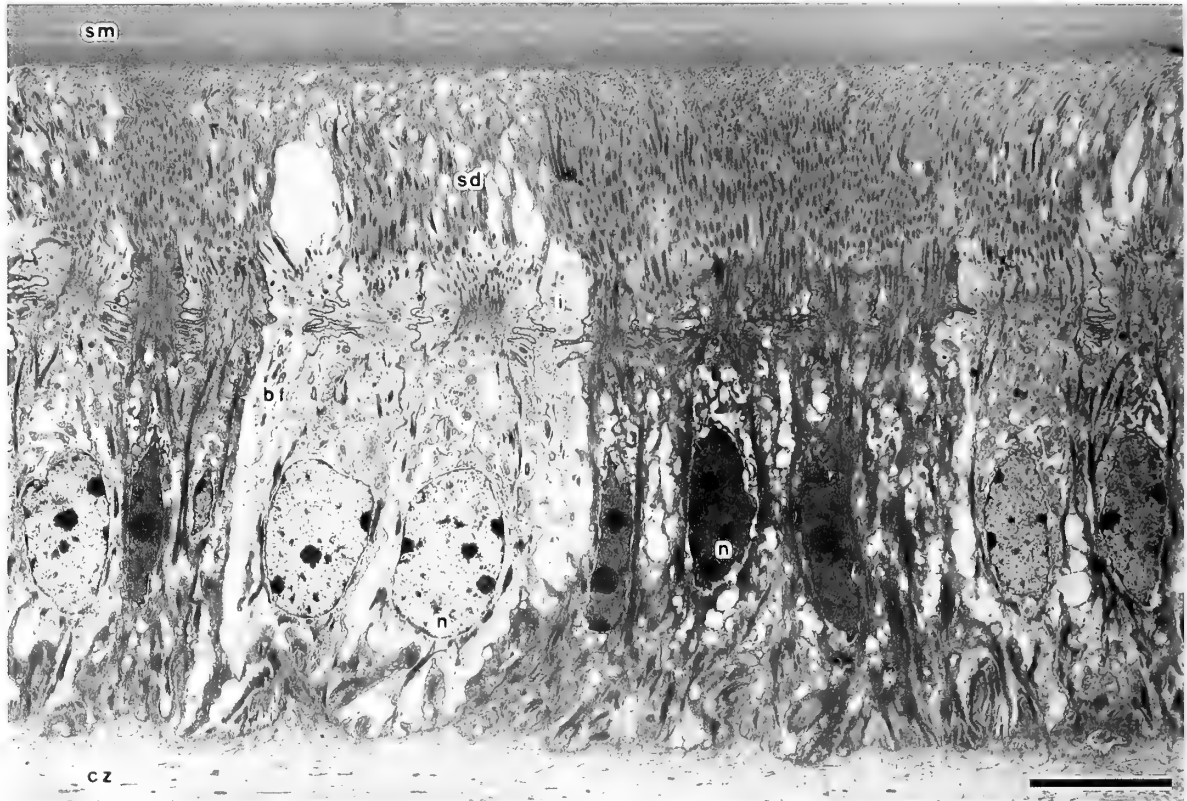


Figure 4. TEM of the myoepithelial cells at the attachment site of the retractor muscle of the adult *Nautilus pompilius*. Key: bf, bundle of fibrils; cz, collagenous zone; i, interdigitation; n, nucleus; sd, secretory droplet; sm, semi-transparent membrane. Scale bar = 10  $\mu\text{m}$ .

This is basically the same as in other molluscan groups such as monoplacophorans (Haszprunar & Schaefer, 1997), gastropods (Tomp & Watabe, 1976), bivalves (Nakahara & Bevelander, 1970), and scaphopods (Shimek & Steiner, 1997). However, *Nautilus* possesses characteristic features in the morphology of the myoepithelial cells and their apical junction to the extracellular sheet (semi-transparent membrane), which is directly attached to the inner wall of the body chamber. The ultrastructures of the myoepithelial cells are illustrated diagrammatically in Figure 2.

#### Adult Stage: Attachment Site of Retractor Muscle

The myoepithelial epithelium at the attachment site of the retractor muscles is connected indirectly with the shell through the medium of the semi-transparent membrane (Figure 3A; sm), which has been variously termed conchin layer, pseudo-tendon (Mutvei, 1957), membranous

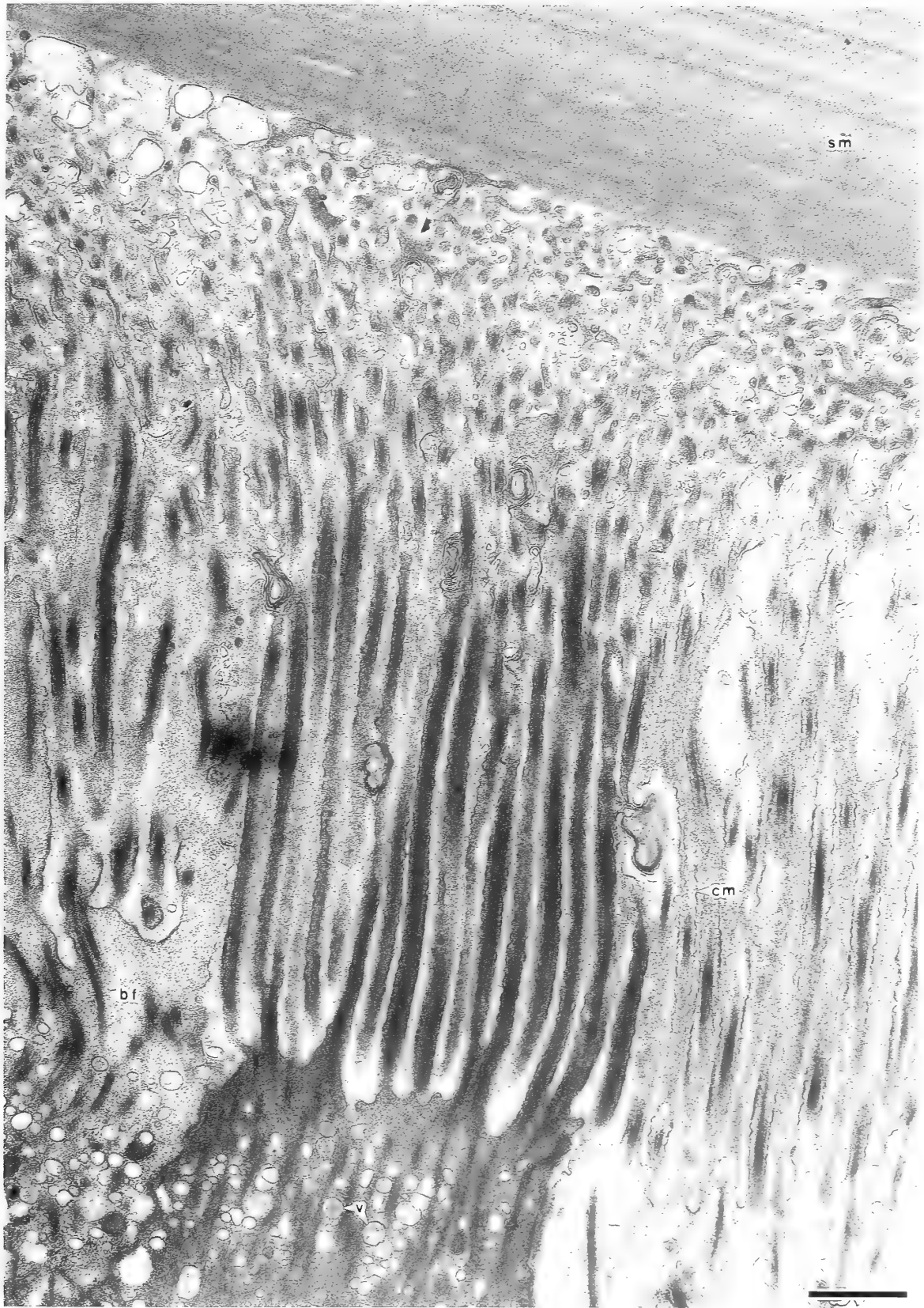
disc (Grégoire, 1962), and an organic (conchiolin) lamella (Mutvei & Doguzhaeva, 1997). Such a membrane attains about 80  $\mu\text{m}$  in thickness and shows a banded structure parallel to the shell surface. It is very similar to the periostracum in electron density (Figures 4, 5).

The myoepithelial cells (height approx. 45  $\mu\text{m}$ , width 6–10  $\mu\text{m}$ ) are high columnar in shape. They have well developed microvilli (length > 10  $\mu\text{m}$ ) which occupy a fourth of the cell height (Figure 4). The basal portion of each microvillus (diameter 0.2–0.25  $\mu\text{m}$ ) is perpendicularly arranged to the apical surface of the cell. Its diameter gradually decreases distally, showing intertwist facing to the inner surface of the semi-transparent membrane (Figure 5). The tips of the microvilli never insert into the membrane. The cytoplasmic membranes of the microvilli are remarkably undulated. Well developed bundles of fibrils occur in the microvilli (Figure 5).

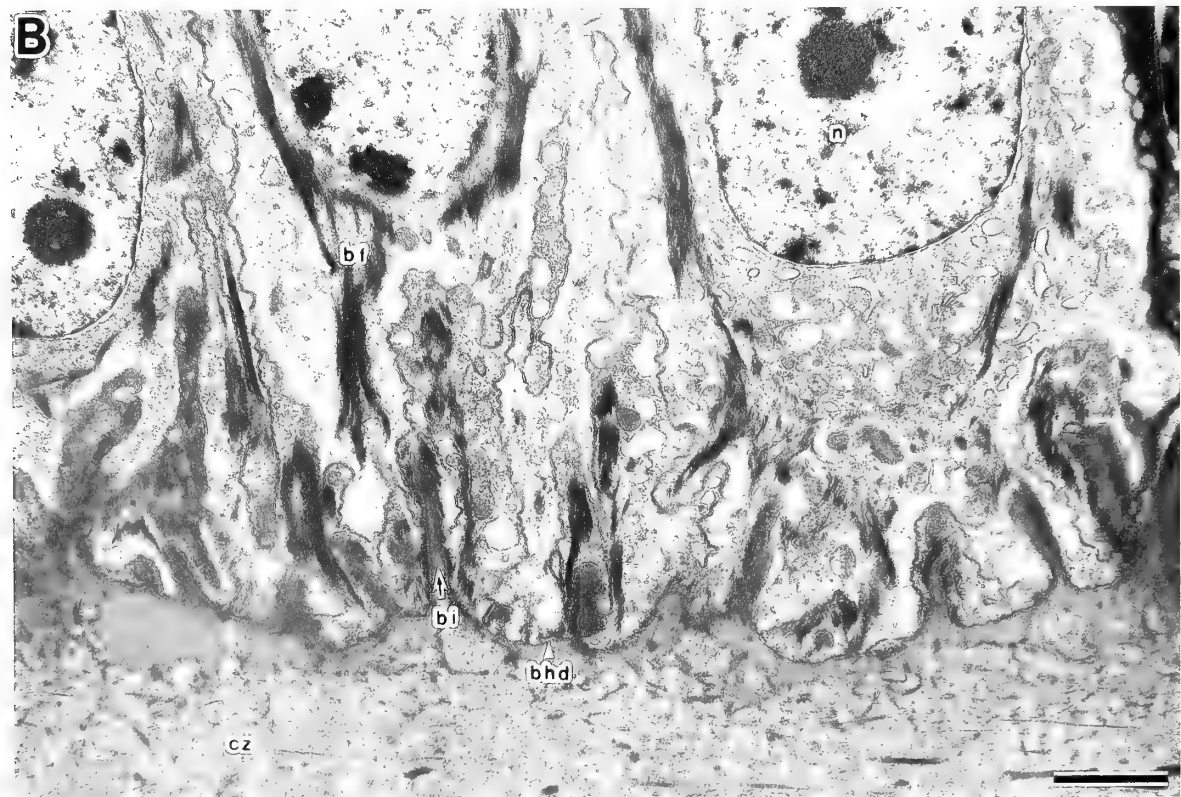
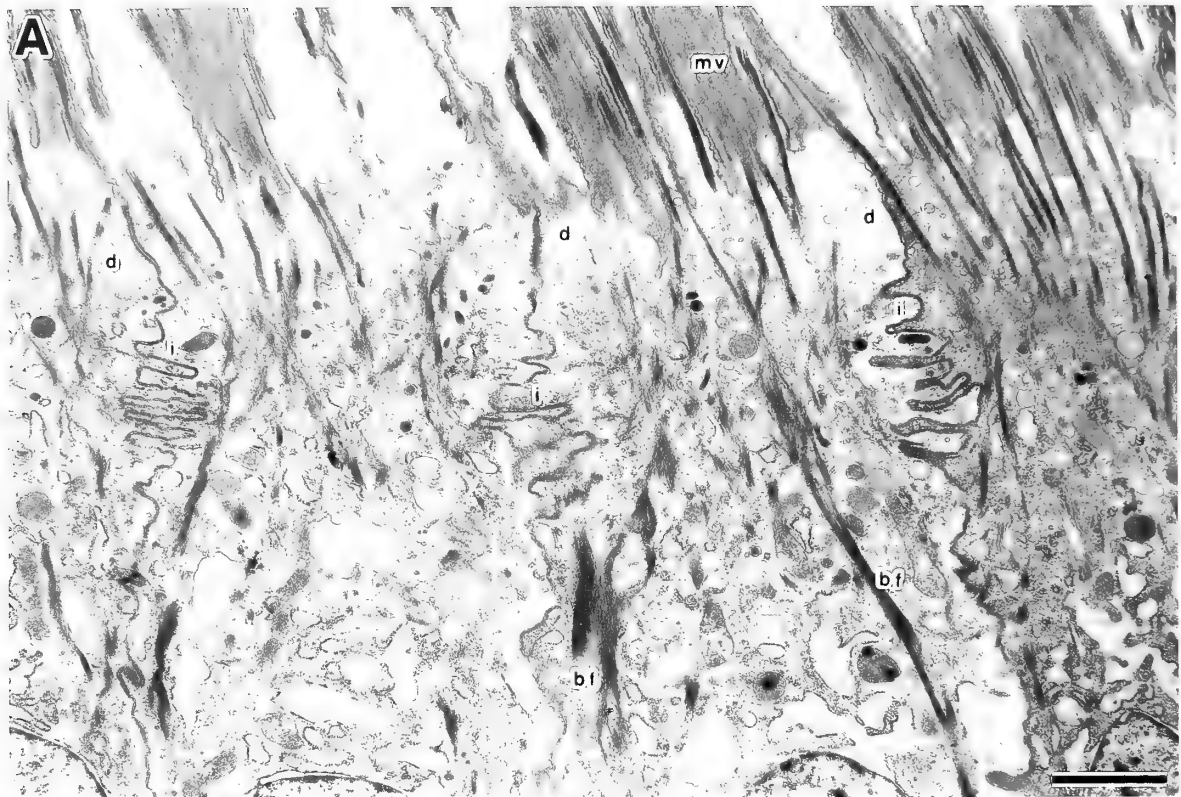
The bundles of fibrils traverse the entire length of the

Figure 5. TEM of the microvilli of myoepithelial cells at the attachment site of the retractor muscle of the adult *Nautilus pompilius*. Key: bf, bundle of fibrils; cm, cytoplasmic membrane of microvilli; sm, semi-transparent membrane; v, vesicle. Scale bar = 1  $\mu\text{m}$ .









cell (Figures 4, 6). They form very large bundles at the central and basal portions of the cell (Figure 6), and split up apically so as to send a bundle to each microvillus (Figures 5, 6A). The bundles of fibrils are basally connected by hemidesmosomes with the basal cytoplasmic membrane (Figure 6B).

Electron densities of the cytoplasm of myo adhesive cells are remarkably variable throughout the epithelium (Figure 4). Numerous secretory droplets produced by electron-lucent epithelial cells are observed in apical and interstitial spaces of the microvilli (Figure 4). Electron-lucent vesicles are especially abundant in the apical portion of the electron-dense cell (Figure 5).

Elongated elliptical nuclei are situated in the basal half of the cell (Figure 4). Their electron densities correspond with those of the cytoplasm. The electron-dense nuclei are more compressed than the electron-lucent nuclei. Each adhesive cell is interconnected to the adjacent cells by belt desmosomes, followed by well developed interdigitations (Figures 4, 6A). Basal infoldings are also well developed in each adhesive cell (Figure 6B).

#### Embryonic Stage: Attachment Site of Retractor Muscle

The myo adhesive epithelial cells at the attachment site of the retractor muscles of the 154-day-old embryo measure about 20  $\mu\text{m}$  in height and 5–10  $\mu\text{m}$  in width. At the ventral edge of the attachment area, the boundary between the adhesive cells and the non-adhesive ones (height about 10  $\mu\text{m}$ ) is recognizable by the drastic change of cell height (Figures 3B, 7). In addition, the microvilli of the most ventrally situated myo adhesive cells are longer than those of the adjacent non-adhesive cells (Figure 7). Toward the dorsal portion of the attachment area, such microvilli gradually increase their length, and their tips become more slender. In association with this change of microvillous length, the cytoskeleton gradually increases, and the cytoplasmic membranes become undulated (Figure 8A).

Dense bundles of fibrils are poorly developed as compared with those of the adult cells. As observed in the adult specimen, the bundles of fibrils split up apically so as to send a bundle to each microvillus (Figure 8A) and are basally connected by hemidesmosomes with the basal cytoplasmic membrane. Elliptical nuclei are situated in the center of the cell. Mitochondria are often concentrated in the upper half of the cell (Figure 8A). Electron density

of the cytoplasm within the myo adhesive epithelium is relatively more uniform in comparison with that of the adult, although there is some variation.

Numerous characteristic projections are observed in the apical surface of the attachment epithelium (Figure 8A, B). Such projections gradually increase in number dorsally within the attachment area, whereas they are absent in the cells near the ventral edge (Figure 7). Each projection is conical in shape, with a slender tip (diameter approx. 0.5  $\mu\text{m}$ ) and has numerous small vesicles (diameter 50–100 nm) and a few cytoskeletons (Figure 8A, B). Interdigitations in the interconnection among the adjacent cells are more poorly developed than those in the adult cells (Figure 8A). Basal infoldings are unclear.

In the attachment site of the retractor muscle of the embryo, the inter- and intra-crystalline organic matrices appear to preserve the shape of the original shell structure. The inner shell wall of the ventral edge of the attachment area is composed of a nacreous layer (= mnw in Tanabe & Uchiyama, 1997) (Figures 3B, 7). In going to the dorsal portion of the attachment area, a prismatic layer (= ipw in Tanabe & Uchiyama, 1997) appears underneath the nacreous layer (Figures 3B, 8B). In accordance with the deposition of the prismatic layer, the innermost shell wall is covered by an organic membrane, which shows a similar electron density to the periostracum (Figure 8B). This organic membrane appears to be the same as the semi-transparent membrane observed in the adult specimens.

The boundary between the nacreous and prismatic layers is clearly marked (Figures 3B, 8B), whereas the boundary between the prismatic layer and organic membrane is irregular because both layers are variable in thickness and in places interfinger with each other (Figure 8B). The wall of the organic membrane facing the apical free surface of the myo adhesive cell is smooth and distinct (Figure 8B).

#### Embryonic Stage: Initial Portion of Siphuncular Cord

The myo adhesive cells at the ventral edge of the initial portion of siphuncular cord are about 20  $\mu\text{m}$  in height and 5  $\mu\text{m}$  in width (Figure 9A). A drastic change of cell height is also observable at the boundary between the non-adhesive and adhesive cells (Figures 3C, 9A).

Characteristic features such as regional variation of the ultrastructure of the microvilli, projections, and interdig-

Figure 6. TEMs of the apical and basal parts of the myo adhesive epithelial cells of the adult *Nautilus pompilius*. A. Bundles of fibrils splitting up so as to send a bundle to each microvillus. Each cell is tightly connected with neighboring cells by well developed interdigitations. B. Bundles of fibrils connected by hemidesmosomes with the basal cytoplasmic membrane which represents basal infoldings. Key: bf, bundles of fibrils; bhd, basal hemidesmosome; bi, basal infolding; cz, collagenous zone; d, desmosome; i, interdigitation; mv, microvilli; n, nucleus. Scale bar = 2  $\mu\text{m}$ .

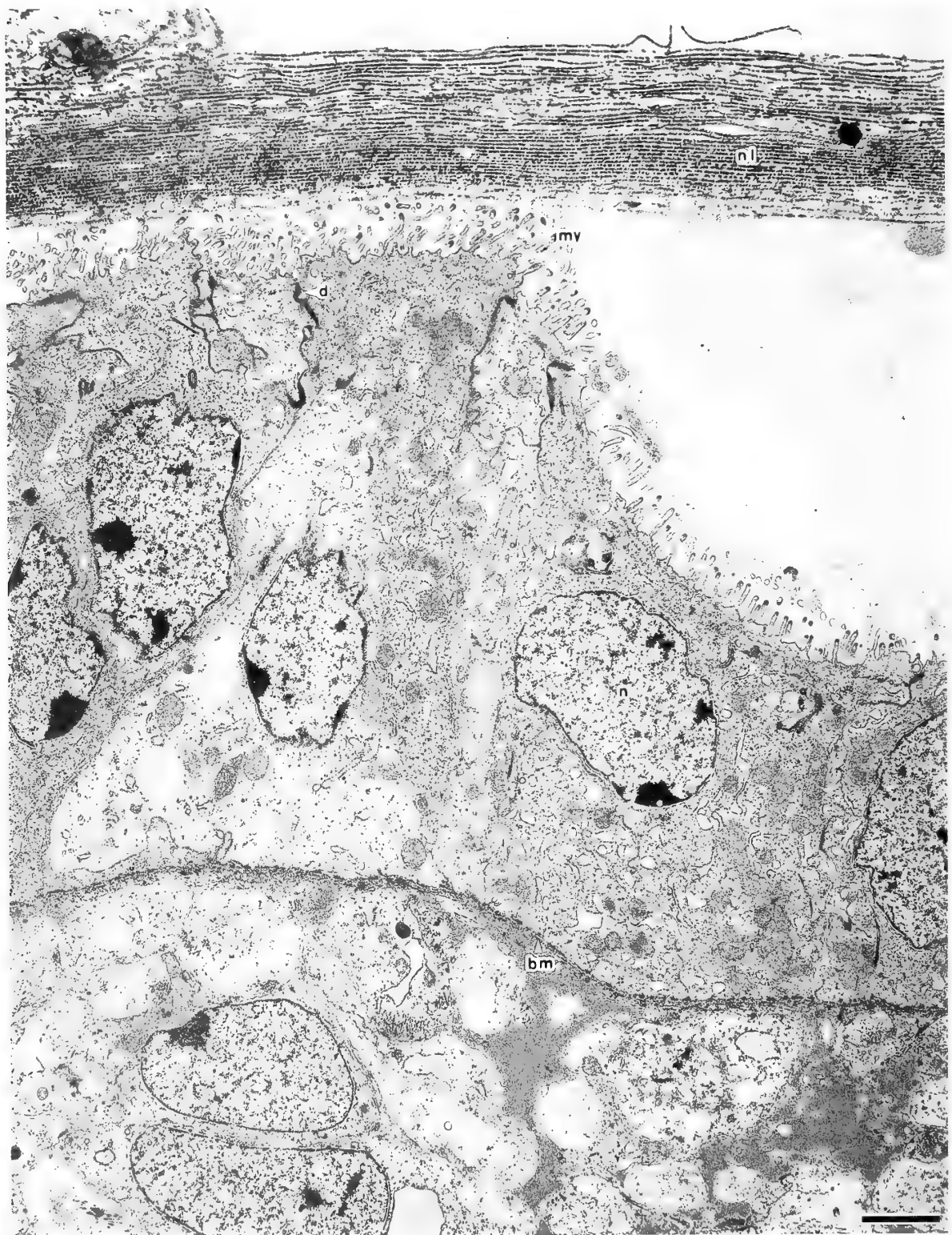


Figure 7. TEM of the ventral edge of the attachment area of the myoepithelium at retractor muscle (asterisk in Figure 3B) showing drastic change of cell height between the ventrally situated non-adhesive cells and the dorsally situated adhesive ones. Key: bm, basal membrane; d, desmosome; mv, microvilli; n, nucleus; nl, nacreous layer. Scale bar = 2  $\mu$ m.

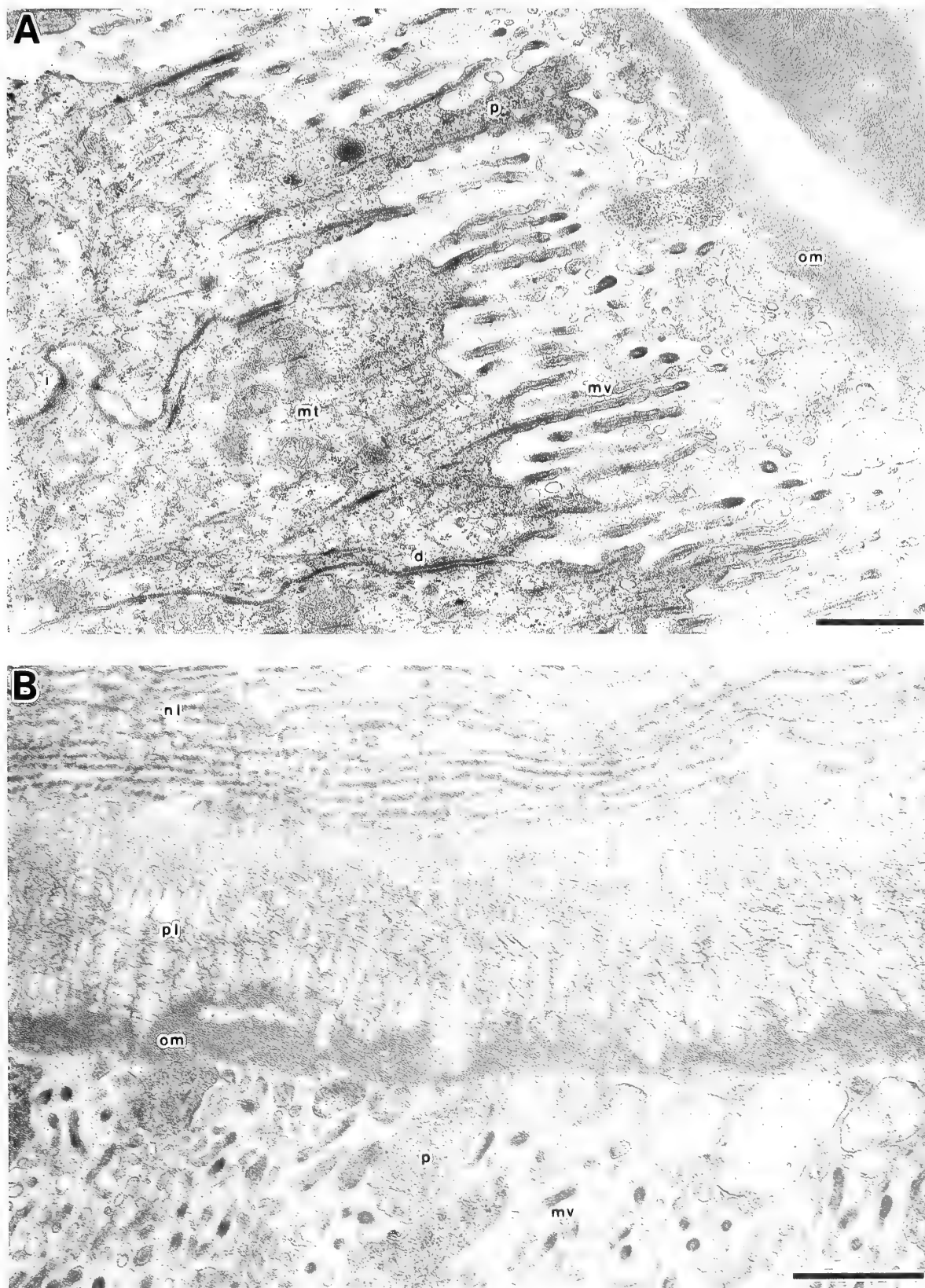
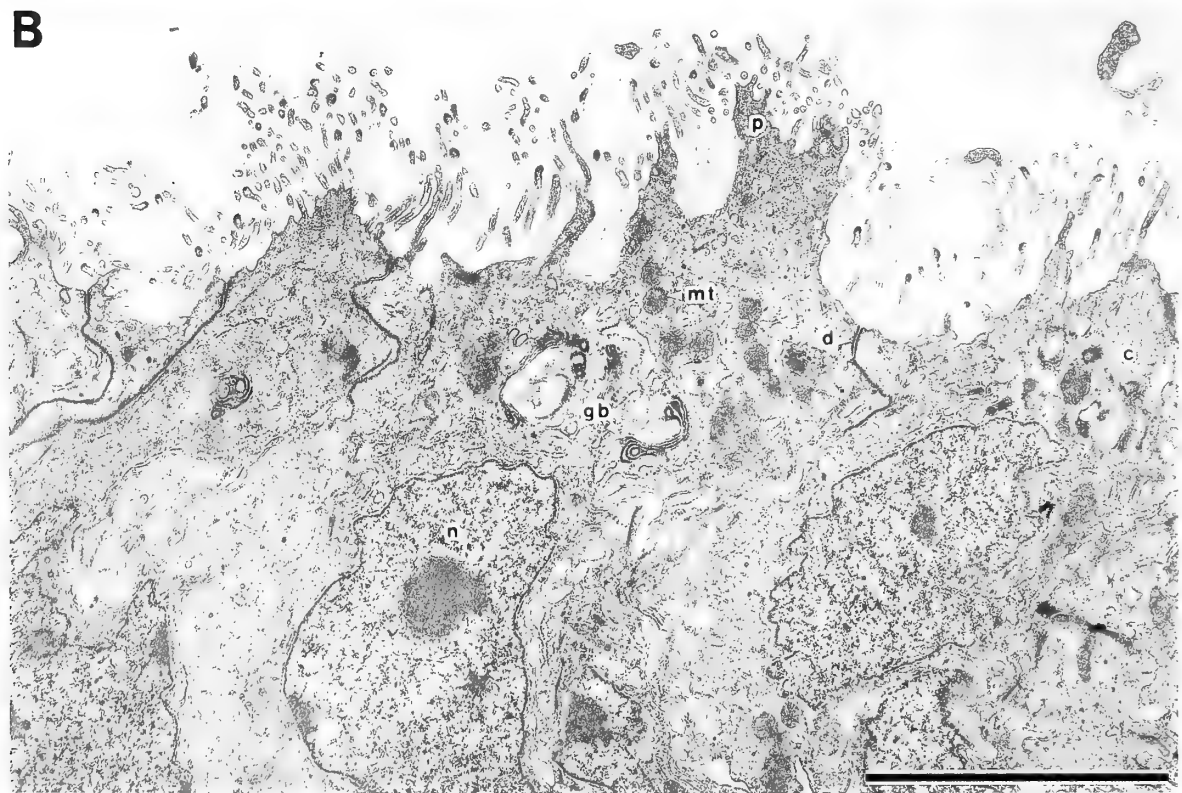
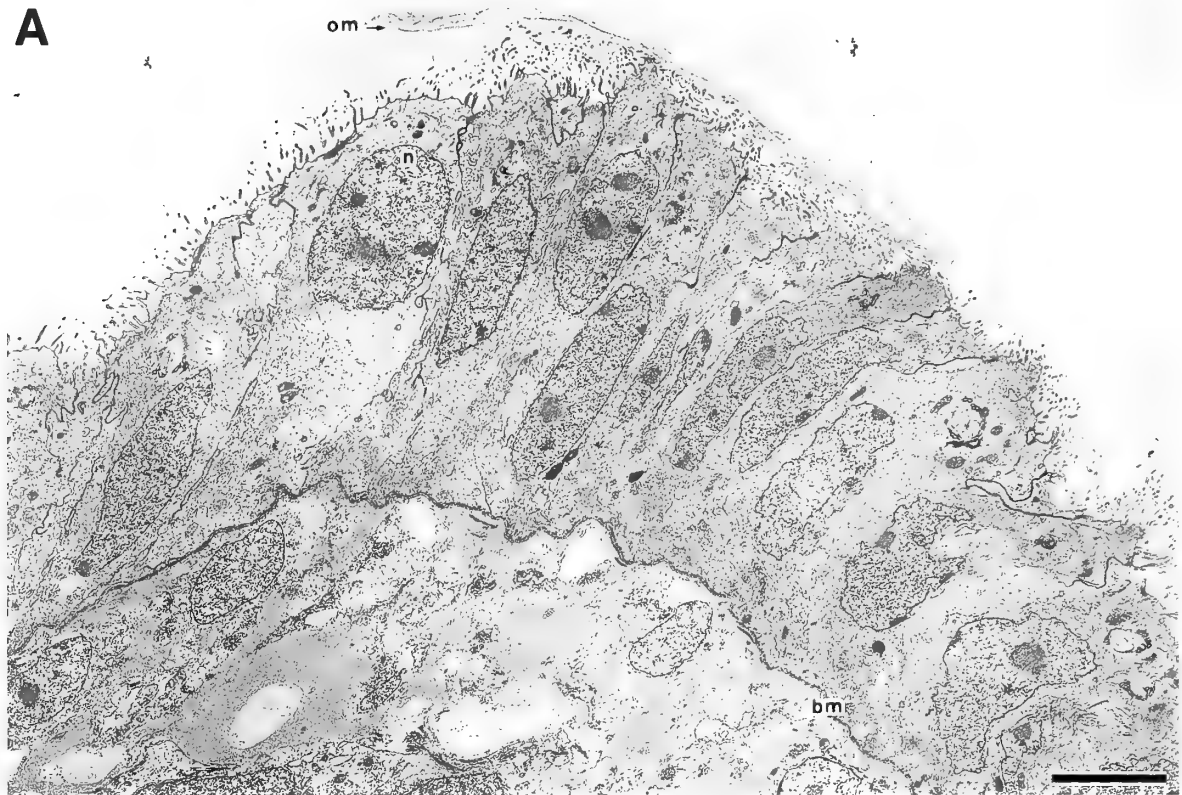


Figure 8. TEMs of the apical portion of the myoaddhesive cells at the retractor attachment site of embryo of *Nautilus pompilius*. A. Myoaddhesive cells showing projections, elongate and undulate microvilli, and weakly developed interdigitation. B. Undissolved organic matrix of nacreous and prismatic layers. Prismatic layer is covered by organic membrane. Key: d, desmosome; i, interdigitation; mt, mitochondria; mv, microvilli; nl, nacreous layer; om, organic membrane; p, projection; pl, prismatic layer. Scale bar = 1  $\mu$ m.





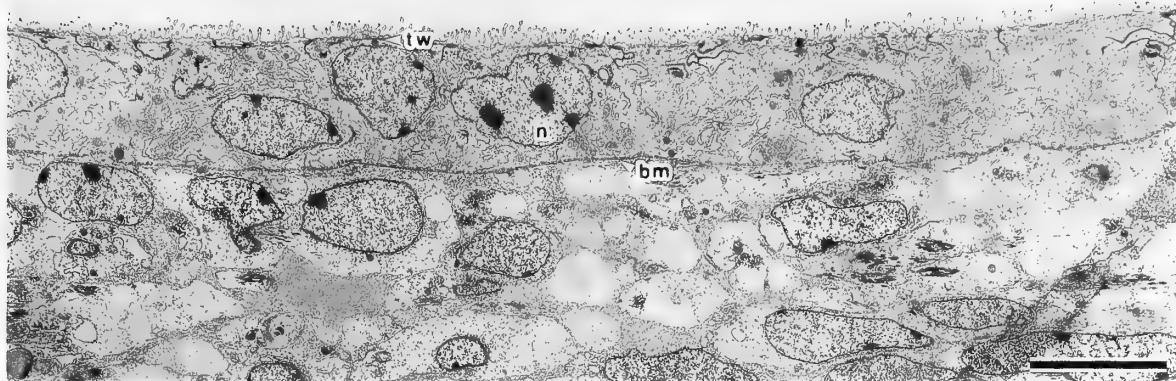


Figure 10. TEM of non-adhesive epithelial cells located near the initial portion of the siphuncular cord of the embryo of *Nautilus pompilius*. Key: bm, basal membrane; n, nucleus; tw, terminal web. Scale bar = 10  $\mu\text{m}$ .

itation within the interconnected cells and the bundles of fibrils are similar to those of the cells at the attachment site of the retractor muscle of the embryo. Golgi bodies and centrioles are observed occasionally, especially in the upper part of the cells (Figure 9B).

At the dorsal edge of the initial portion of the siphuncular cord, the height of the adhesive cells is the same as that of the adjacent non-adhesive ones, whereas the microvilli are not elongated and the projections do not occur in the non-adhesive cells. A few extracellular matrices are observed at the ventral edge of the myo adhesive epithelium (Figure 9A). It is not clear whether these matrices are of undissolved shell origin or represent an organic membrane facing the inner shell wall.

#### Non-Adhesive Epithelium (Simple Mantle Epithelium)

Non-adhesive epithelial cells are observed at three different epithelial portions of the embryo: the area in front of the ventral edge of the retractor attachment site, and the adjacent sites of the ventral and dorsal edges of the initial portion of the siphuncular cord. These cells are shorter (height approx. 10  $\mu\text{m}$ ) than those of the adjacent adhesive cells (Figure 3B, C). The terminal webs are well developed at the apical portion (Figure 10). They do not exhibit specific features such as elongate microvilli with undulate cytoplasmic membrane, projection, interdigitation, and bundles of fibrils, all of which occur in the adhesive cells.

## DISCUSSION

### Ultrastructural Features of Muscle-Shell Attachment

As already described by previous workers (e.g., Mutvei, 1957; Grégoire, 1962) in Recent *Nautilus*, a thick semi-transparent membrane lines the inner surface of the shell wall of the body chamber in front of the last septum. Our TEM observations have revealed that the membrane at the attachment site of the retractor muscle in the embryo has an irregular surface, seemingly providing a firm attachment to the prismatic shell layer of the inner shell wall. Although we could not observe the ultrastructure of the shell-membrane junction in the adult specimens, the same relationship observed in the embryo may exist because the texture of the inner surface of the shell wall just beneath the membrane exhibits "swarming lenticular and spheroidal seed crystallites" (Grégoire, 1962, 1987: 472) and "vertically oriented acicular crystallites" (Mutvei & Doguzhaeva, 1997:48). In addition, such texture differs greatly from that of the membrane-free inner shell surface (Grégoire, 1962, 1987). Therefore, the surface texture of the attachment scars appears to be effective for a firm attachment of the shell to the membrane.

Judging from the ultrastructure of the shell-membrane junction, the shape of the scar produced on the internal shell wall should correspond to the attachment area of the semi-transparent membrane, which appears to be secreted by the myo adhesive epithelium. Thus, the attachment area

Figure 9. TEMs of the attachment site of the initial portion of the siphuncular cord in the embryo of *Nautilus pompilius*. A. Boundary between myo adhesive (right) and non-adhesive (left) cells at the ventral edge of the attachment area (asterisk in Figure 3C). B. Enlarged view of the right corner of Figure 9A, showing projections and elongate microvilli. Key: bm, basal membrane; c, centriole; d, desmosome; gb, Golgi body; mt, mitochondria; n, nucleus; om, organic membrane; p, projection. Scale bar = 5  $\mu\text{m}$ .

of this membrane also should correspond to the myoadhesive epithelial area. Curiously, the attachment area of the myoadhesive cells occurs beyond the posterior edge of the lateral termination of the retractor muscle (Figure 1). Such a situation has not been reported in other mollusks. This fact simply indicates that it is impossible to reconstruct the exact details of the shape of the lateral termination of the muscle based on the attachment scar, if other externally shelled cephalopods in the fossil record have a similar mode of shell-muscle attachment as is observed in *N. pompilius*.

This study also revealed that the tips of elongate microvilli do not insert into the membrane and have no specific features showing a firm connection with the membrane. Therefore, there may be a unique interconnection by means of an indirect process between the two. Dense slender microvilli that are intertwined with each other appear to form a distinct plane at the free surface. There might be little interstitial space between the microvillous plane and the inner surface of the membrane. If the interstitial space of microvilli is filled with fluid, it might produce an adhesive property between the two planes.

The myoadhesive cell in adult *Nautilus* has characteristic features such as a tightly packed high columnar shape, interconnection by well developed interdigitations, bundles of fibrils, and basal infoldings, suggesting that the epithelium has sufficient strength to resist the tension produced by muscle movement. Furthermore, the association of interdigitations, basal infoldings, and elongate microvilli also suggests that the myoadhesive cells have an active property of ion transport which may be involved in the secretion of the semi-transparent membrane or some kind of fluid. These features are also observed but poorly developed in the myoadhesive cells of the embryo. This fact suggests that the development of myoadhesive cells occurs after hatching in relation to the increase of muscle movement for swimming and some kinds of locomotion, although differentiation of myoadhesive cells begins even at the relatively static embryonic stage.

Cell projection is a unique feature in the myoadhesive cell of the embryo. It might gradually disappear with growth. Its functional significance is unknown. According to Mutvei & Doguzhaeva (1997), the inside surface of the adult shell aperture of *N. pompilius* is perforated by vertical canals, in which finger-shaped epithelial extensions from the mantle are presumably inserted. Mutvei & Doguzhaeva (1997) suggested that the mantle seems to be firmly attached to the apertural region of the shell. In the embryonic stage, however, the projections do not insert into the shell. Thus, it is not plausible that the projections are homologous to each other.

In summary, the myoadhesive cell appears to have a physically weak junction at the apical free surface with the semi-transparent membrane, but the epithelium itself is sufficiently strong to resist the tensile stress caused by

muscle movement. The method for muscle attachment to the shell of *Nautilus* is unique as compared with those in other examined mollusks. According to Tompa & Watabe (1976), in gastropods, the myoadhesive cell (Tompa & Watabe's tendon cell) is attached to the tendon sheath by means of hemidesmosomes at the tips of their very short microvilli, and the tendon sheath inserts fibers into the shell during calcification. The method for muscle attachment to the shell and the ultrastructure of myoadhesive epithelium observed in gastropods are the same as those in monoplacophorans (Haszprunar & Schaefer, 1997), scaphopods (Shimek & Steiner, 1997), and bivalves (e.g., Nakahara & Bevelander, 1970). Such a method of muscle-shell attachment seems to be physically stronger against some kinds of tension than is the case in *Nautilus*.

Bandel & Spaeth (1983) pointed out the morphological similarities between the myoadhesive and siphuncular epithelia of *Nautilus* on the basis of light microscopy. The siphuncular epithelium of *Nautilus* functions as pumping involved in emptying the cameral fluid from the air chambers into blood vessels (Denton & Gilpin-Brown, 1966; Greenwald et al., 1982). The siphuncular epithelial cells form an extensive system of basolateral cell infoldings (canaliculi) that are lined with mitochondria and give the cytoplasm a feathery appearance (Greenwald et al., 1982). However, the myoadhesive epithelium does not possess canaliculi, and the number of mitochondria in each cell is less than that in the siphuncular epithelium.

In addition, the apical portion of the myoadhesive epithelium of *N. pompilius* resembles the siphuncular epithelium of *Sepia officinalis* Linnaeus, 1758, in the presence of elaborate microvilli underneath the organic sheet that closes the cuttlebone posteriorly (Wendling, 1987, cited in Budelmann et al., 1997:fig. 55). In both *Nautilus* and *Sepia*, the apical infoldings occur in the siphuncular epithelial cells, but they do not occur in the myoadhesive cells of *Nautilus*. Therefore, the ultrastructural similarities of the myoadhesive and siphuncular epithelial cells between *Nautilus* and *Sepia* appear to have originated in the high activity of ion transport.

### Functional Aspects of Muscle-Shell Attachment

As described above, the ultrastructure of the muscle-shell attachment in *Nautilus* is unique and differs from those of other mollusks. Interestingly, the ultrastructural characteristics observed in the muscle-shell attachment in the latter groups are essentially similar to those of the buccal muscle attachment to the beak in octopods and squids (Dilly & Nixon, 1976), the muscle attachment to the shell of articulated brachiopods (Stricker & Reed, 1985), and the peduncle muscle attachment to the cuticular flange in the opercular filament of polychaete annelids (Bubel, 1983). In polychaete annelids, for example, the elongate specialized microvilli of the myoadhesive cells penetrate the inner wall of the cuticular flange, sug-



gesting that an extremely tight connection occurs between the epithelium and cuticular flange. These examples suggest that a tight connection occurring at the muscle attachment to the hard exoskeleton is a common condition among different animals.

Why does *Nautilus* develop such a curious muscle-shell attachment system? How is it effective in relation to the mode of life and ontogenetic growth? The nektonic mode of life in *Nautilus* may be a key to assuming these questions. Most other externally shelled mollusks are benthic. When benthic mollusks perform various actions such as valve opening and closing, or crawling on and burrowing into substratum, they bear their own weight on the muscular system. In such situations, the myoadhesive epithelium functions as tendon because it receives a large tensile stress caused by muscle movement. In other words, in benthic mollusks, a tight connection between the internal shell wall and the myoadhesive cells appears to be necessary to sustain the large tensile stress. On the contrary, *Nautilus* maintains neutral buoyancy in the water column by means of a chambered shell filled with low-pressure gas and small amounts of liquid. Therefore, the tensile stress bearing the myoadhesive cells may be relatively reduced in this animal.

Another factor is the mode of mantle shifting through the inside of the body chamber during growth. In *Nautilus*, the formation of a new chamber is episodic. At the stage of a new chamber formation, the septal myoadhesive band is rapidly moved forward in the body chamber, where it reattaches to the internal shell wall (Ward & Chamberlain, 1983). It is suggested that a loose connection between the epithelium and the semi-transparent membrane lining the inner wall of the body chamber may be effective for the alternating mode of peeling off and reattachment of the septal myoadhesive band. By contrast, the shift of the attachment site for the retractor muscle corresponds to the apertural shell growth, which is constant throughout new chamber formation (Ward & Chamberlain, 1983). However, there are no structural differences between the retractor and septal myoadhesive cells based on light microscopic observations. Having a loose attachment of the epithelium to the shell might also be a favorable condition for moving of the myoadhesive cells because they appear to shift more than 30 centimeters throughout ontogeny, whereas the expansion rate of the lateral termination of the muscle seems to be considerably smaller as compared with that of other mollusks.

As pointed out above, the nektonic mode of life and the mode of shell growth in combination with the chamber formation cycle during ontogeny in *Nautilus* are unique among mollusks. Therefore, the muscle-shell attachment in *Nautilus* might have developed as a response to its own functional demands. If the mechanism of muscle-shell attachment in mollusks is highly constrained by mode of life and shell growth, a similar mechanism might

occur even in distantly related taxa. In fact, all mollusks hitherto examined excluding *Nautilus*, are sessile or mobile benthos. Thus, there is a possibility that nektonic and planktonic mollusks such as janthinid, pteropod, and heteropod gastropods have a similar mechanism of muscle-shell attachment to that observed in *Nautilus*.

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## Range Extensions of Sacoglossan and Nudibranch Mollusks (Gastropoda: Opisthobranchia) to Alaska

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**Abstract.** Range extensions to or within Alaska are described for 16 species of opisthobranch mollusks. The ranges of nine of these, including the sacoglossans *Alderia modesta* and *Olea hansineensis* and the arminacean nudibranch *Janolus fuscus*, are extended northward from British Columbia or southeast Alaska. The ranges of another five species, including four dorids and the arminacean *Armina californica*, are extended westward from sites within Alaska. The range of an arctic species, *Calycidoris guentheri*, is extended southward into the central Bering Sea, and that of the circumboreal *Palio dubia* northeastward to south-central Alaska. Given the circumboreal or amphi-Pacific distributions of about half of these species, as well as the paucity of previous observations in much of Alaska, we consider most, if not all, of these range extensions the result of increased or fortuitous search efforts, rather than actual range expansions by the species themselves.

### INTRODUCTION

Lee & Foster (1985) reviewed the literature and summarized the known records of opisthobranchs from Alaska, greatly expanding our knowledge of the fauna from the Gulf of Alaska, Bering Sea, and Arctic Ocean. More recently, Foster (1987a, b), Millen (1989), Behrens (1997, 1998), and Goddard (2000) extended the ranges of additional species to southeast and south-central Alaska; and Millen (1985, 1987) synonymized several species of onchidoridid nudibranchs reported from Alaska. Since these reports, we have continued our studies of the opisthobranch fauna of Alaska, focusing especially on sites on the Kenai Peninsula and in Prince William Sound during surveys of non-indigenous marine species led by researchers from the Smithsonian Environmental Research Center (SERC) (Hines et al., 2000). In addition, we have examined specimens collected by other researchers and deposited in the University of Alaska Museum since 1985. As a result of these studies, we have extended the known ranges of two sacoglossans and 14 nudibranchs to or within Alaskan waters. This paper documents these range extensions and discusses some of their biogeographic implications.

### STUDY SITES AND METHODS

We searched for opisthobranchs at sites on the Kenai Peninsula and in Prince William Sound (Figure 1, Table 1), during June and September 1998, August 1999, and July

2000. We collected specimens by hand from intertidal mudflats and rocky shores, floating docks and buoys, rock jetties, and from settling plates deployed in the shallow subtidal by personnel from SERC earlier in the year. We used a dissecting microscope or hand lens to observe specimens alive, and then fixed them in either 5 to 10% formalin or 70% ethanol. Specimens not identified in the field, or those collected by other workers and already deposited in the University of Alaska Museum, were examined, dissected, and identified in the laboratory at either the University of California at Santa Barbara or the University of Alaska in Fairbanks. We deposited voucher specimens in the University of Alaska Museum (UAM) in Fairbanks or in the California Academy of Sciences invertebrate zoology collection (CASIZ) in San Francisco. Catalogue numbers for these are given with the species accounts below.

### RESULTS

We extended the known ranges of 16 species: nine species northward from British Columbia and southeastern Alaska, five species westward from sites within Alaskan waters, one species northeastward from the Aleutian Islands, and one species southward within the Bering Sea. These species are listed below (alphabetically within each higher taxon) with notes on their classification, habitats, and prey.

In addition to the following species, we also found *Cuthona albocrusta* (MacFarland, 1966) on floating docks

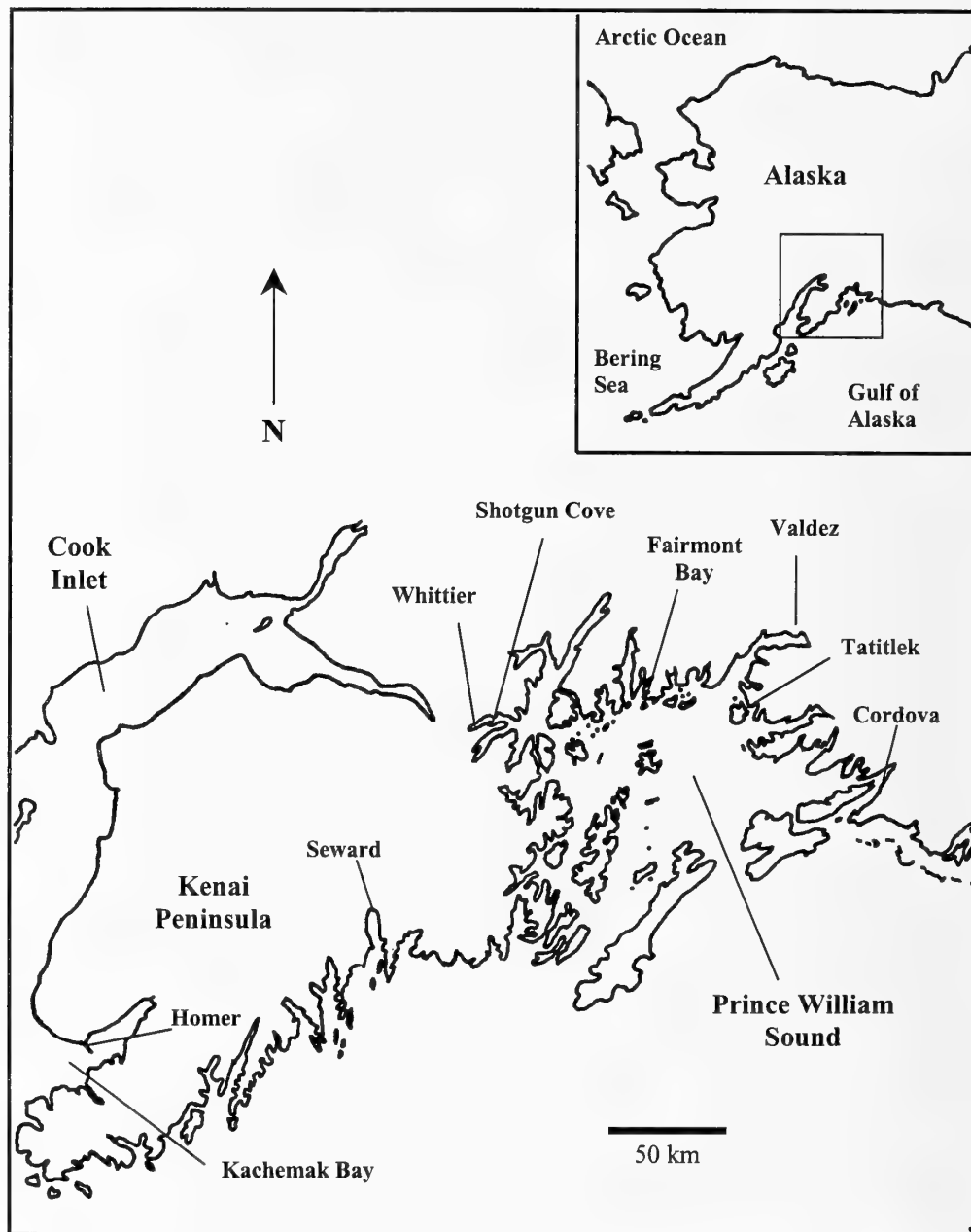


Figure 1. Map of the Kenai Peninsula and Prince William Sound, Alaska, showing location of the study sites.

in Cordova, Prince William Sound (Figure 1). Goddard (2000) reported this range extension from southern British Columbia, but we only recently deposited the specimen on which that range extension was based in the California Academy of Sciences (CASIZ 146069).

#### Sacoglossa

##### *Alderia modesta* (Lovén, 1844)

Adults and their egg masses were abundant on the yellow-green alga *Vaucheria* sp. on the high intertidal mud-

flats immediately southwest of the Cordova marina on 13 August 1999. We have deposited three specimens in the California Academy of Sciences (CASIZ 142448). This is a range extension of 1620 km northwest from Port Alice, Vancouver Island, British Columbia (Millen, 1980).

Millen (1980:1209) noted that *Alderia albopapillosa* Dall, 1871, collected by Dall (1871) from Sitka, Alaska, might be synonymous with *A. modesta*. However, as pointed out by Hand & Steinberg (1955:26), Bergh

Table 1

Location of study sites for opisthobranch gastropods in south-central coastal Alaska.

Site	Latitude (N)	Longitude (W)
Cordova	60°32.50'	145°46.47'
Fairmont Bay	60°53.05'	147°23.52'
Hanning Bay, Montague Island	59°57'	147°46'
Homer spit marina	59°37.97'	151°26.17'
Little Takli Island	58°04'	154°29'
Port Etches, Hinchinbrook Island	60°13'	146°42'
Seward	60°07.43'	149°22.72'
Shotgun Cove	60°47.43'	148°32.50'
Tatitlek	60°52.10'	146°43.47'
Valdez	61°07.42'	146°21.25'
Whittier	60°46.62'	148°41.40'

(1880) showed that Dall's specimens of *A. albopapillosa* actually belonged to the dorid nudibranch genus *Adalaria*. Millen (1987) later synonymized *Adalaria albopapillosa* (Dall, 1871) with *Adalaria proxima* (Alder & Hancock, 1854), but did not mention its previous and short-lived placement in the genus *Alderia*.

#### *Olea hansineensis* Agersborg, 1923

One specimen (CASIZ 142449) of this opisthobranch egg-eating sacoglossan was found on an egg mass of the cephalaspidean *Melanochlamys diomedea* (Bergh, 1894) on the mudflat southwest of the Cordova marina on 13 August 1999. This is a range extension of 1825 km northwest from Sechart Inlet, British Columbia (Millen, 1980).

#### Nudibranchia, Doridacea

##### *Adalaria jannae* Millen, 1987

This species was abundant, along with its ribbon-shaped egg masses, on the encrusting bryozoan *Membranipora* sp. growing on the kelp *Laminaria* sp. on floating docks at Whittier and on a moored buoy in Shotgun Cove, both on 10 August 1999. Owing to the influence of glaciers and snow fields in the fjord surrounding Whittier, the salinity (and temperature) of the surface water in the marina was very low, and we found nudibranchs, their prey, and other fouling invertebrates at this site only on *Laminaria* growing below the fresh water lens. *Adalaria jannae* closely resembles *Onchidoris muricata*, but lacks the medial radular teeth found in the latter; *A. jannae* also has four to six small lateral teeth on each half row of the radula, as well as ribbon-shaped egg masses (Millen, 1987). The radular formula from an 8 mm-long (preserved) specimen from Shotgun Cove was 30 × 4.1.0.1.4. Eight specimens from Whittier have been deposited in the California Academy of Sciences (CASIZ 142450). This

is a range extension 1745 km northwest from Sointula, British Columbia (Millen, 1987).

##### *Adalaria* sp. 1 of Behrens (1991) and Millen (1987:2701)

One specimen (CASIZ 142451), 3.3 mm long (preserved) was found on the low intertidal rocky shore at Tatitlek on 12 August 1999. The morphology of this specimen matched that of specimens observed by one of us (JHRG) in Oregon and Washington (Goddard, 1984, Goddard et al., 1997). This is a range extension of 1080 km northwest from Ketchikan, Alaska (Millen, 1989).

##### *Ancula pacifica* MacFarland, 1905

A single specimen (CASIZ 142452), lacking orange lines on the body, was found on the floating docks in the Cordova marina on 13 August 1999. This species (or just the color form of *A. pacifica* lacking orange lines on the body) may be a junior synonym of *Ancula gibbosa* (Risso, 1818), which is known from the north Atlantic Ocean and Barents Sea (McDonald, 1983; Thomson & Brown, 1984). This is a range extension of 1080 km northwest from Grant Island, Ketchikan, Alaska (Millen, 1989).

##### *Archidoris odhneri* (MacFarland, 1966)

One specimen (UAM 7153) was collected from unknown depth by R. Baxter 31 January 1985 using a bottom trawl on the continental shelf off the north side of the Alaska Peninsula (57°00'N, 162°03.40'W) in the Bering Sea. This is a range extension of 660 km southwest from Port Dick on the Kenai Peninsula, Alaska (Robilliard & Barr, 1978).

##### *Calycidoris guentheri* Abraham, 1876

One specimen (UAM 7154) was collected from unknown depth by R. Baxter 2 September 1985 using a bottom trawl on the continental shelf in the central Bering Sea (57°17.68'N, 178°20.15'W). This is a range extension of 1250 km southwest from the Bering Strait (Lee & Foster, 1985).

##### *Diaulula sandiegensis* (Cooper, 1863)

One specimen (UAM 7155) was collected from unknown depth by R. Baxter 9 October 1986 using a bottom trawl in the central Aleutian Islands (52°21.44'N, 179°49.23'W). This is a range extension of 850 km west from Unalaska Island (Bergh, 1894).

##### *Doridella steinbergae* (Lance, 1962)

Foster (1987a) extended the range of this species from Bamfield, Vancouver Island, British Columbia to Prince William Sound, Alaska. During the present study we found 12 specimens of *Doridella steinbergae*, 1 to 5 mm

long, on its prey, *Membranipora* sp., on drift kelp *Laminaria* sp. on the mudflats at Cordova on 13 August 1999 (CASIZ 146077). We also found this species on *Laminaria* sp. in the low rocky intertidal zone at Little Takli Island, Katmai National Park on 27 July 1998, extending its range 475 km southwest from Prince William Sound (Foster, 1987a).

*Geitodoris heathi* (MacFarland, 1905)

Four specimens were found on the low intertidal rocky shore at Tatitlek on 12 August 1999; three of these have been deposited in the California Academy of Sciences (CASIZ 142453). This is a range extension of 1080 km northwest from Ketchikan, Alaska (Millen, 1989).

*Palio dubia* (M. Sars, 1829)

Adults and egg masses (CASIZ 142454) were abundant on the bryozoan *Membranipora* sp. growing on the kelp *Laminaria* sp. on the floating docks at Whittier on 10 August 1999. Additional specimens were found on the buoy at Shotgun Cove on 10 August 1999 and on the docks at Cordova on 13 August 1999. Our specimens were uniformly translucent light brown in color, with delicate and flaccid bodies, and had five branchial plumes. The rhinophores on one specimen had 12 lamellae. The radula from another specimen had a formula of  $15 \times 5.2.0.2.5$ , with the marginal teeth ranging in size from (inner to outermost) 135 to 45  $\mu\text{m}$  high by 50 to 10  $\mu\text{m}$  wide. These characters closely match those of *P. dubia* described by Thompson & Brown (1984) and Picton & Morrow (1994). They also match Bergh's (1880) description of *P. pallida*. Thompson & Brown (1984) consider the latter a junior synonym of *P. dubia*; we concur. The specimens from Cordova represent a range extension of 2450 km northeast from Kiska Island in the Aleutians (Bergh, 1880). *Palio dubia* has also been recorded from the northern Sea of Japan (Martynov, 1998a).

*Triopha catalinae* (Cooper, 1863)

One specimen (UAM 7156) was collected by C. Simenstad from 6 m depth on 30 June 1987 off Shemya Island (52°43.33'N, 174°07.00'E) in the Aleutians. This is a range extension of 360 km northwest from Amchitka Island (Robilliard, 1974).

Nudibranchia, Arminacea

*Armina californica* (Cooper, 1863)

One specimen (UAM 7157) collected from unknown depth by R. Baxter on 23 September 1986 using a bottom trawl off the north side of Akutan Island (54°16.9'N, 165°57.09'W) in the Aleutian Islands. This is a range extension of 1450 km southwest from Kayak Island in the Gulf of Alaska (Lee & Foster, 1985). Additional speci-

mens (UAM 7158 & 7159) were collected by one of us (NRF) in March 1986 from between 102 and 140 m depth in Hanning Bay, Montague Island and between 55 and 129 m in Port Etches, Hinchinbrook Island. Both of these latter sites are in Prince William Sound (Table 1).

*Janolus fuscus* (O'Donoghue, 1924)

Two specimens, 60 and 70 mm long, of this distinctive species were found with their egg masses on the bryozoan *Bugula* sp. on floating docks in the Homer marina at the mouth of Kachemak Bay on 8 August 1999. We returned these specimens to their habitat after making our observations, confirming their identity, and showing them to others members of the SERC survey team. This is a range extension of 1250 km northwest from Klu Bay, Revillagigedo Island, southeast Alaska (Robilliard & Barr, 1978).

Nudibranchia, Aeolidacea

*Cuthona pustulata* (Alder & Hancock, 1864)

Four specimens (CASIZ 146070), 4 to 5 mm long, were found feeding on the hydroid *Sarsia* sp. on a dock in the marina at Homer on 8 August 1999. These specimens resembled Gosliner & Millen's (1984) description of *Cuthona pustulata* from British Columbia, especially with regard to overall shape of the body, cerata, and head tentacles. However, our specimens were smaller than those examined by Gosliner & Millen (1984) and differed by lacking large white spots on the cerata (they did have smaller opaque white flecks). They also had slightly fewer rows of cerata (six to eight compared to eight to 14), with fewer cerata per row (one to three, compared to two to eight reported by Gosliner & Millen (1984) for a 16 mm-long specimen). The radula and shape of the radular teeth of our specimens were virtually identical to that described by Gosliner & Millen (1984) but differed in having four to five lateral denticles, instead of five to nine. These specimens represent a range extension of 2160 km northwest from Galiano Island, British Columbia (Gosliner & Millen, 1984).

*Eubranchius olivaceus* (O'Donoghue, 1922)

We found 10 specimens with their egg masses on the hydroid *Obelia* sp. growing on floating docks in the Homer marina on Kachemak Bay on 8 August 1999 (CASIZ 146071). We found an additional specimen on *Obelia* sp. on docks at Whittier on 10 August 1999. The body of these specimens was translucent with small epidermal flecks of either encrusting white or encrusting reddish brown. The cerata, but not the rhinophores or cephalic tentacles, had a subterminal band of encrusting brown pigment. On some specimens, encrusting white pigment was concentrated distally on the rhinophores and cerata. The cerata cores and the branches of the digestive gland

in the body were green to greenish brown, imparting an overall greenish hue to the body. The coloration of these specimens was virtually identical to that observed by Goddard et al. (1997) in specimens from *Obelia* sp. in Neah Bay, Washington. The specimens from Homer represent a range extension of 970 km west from Amalga Harbor near Juneau in southeast Alaska (Behrens, 1997). *Eubbranchus olivaceus* has also been reported from Ketchikan, Alaska (Millen, 1989).

As described in Just & Edmunds (1985:114), Henning Lemche considered *Eubbranchus olivaceus* very similar to, if not synonymous with, *E. rupium* (Möller, 1842) from the north Atlantic Ocean. Martynov (1998b) synonymized the former with the latter; he also erected a new genus, *Nudibranchus*, to include *E. rupium* and some other species of *Eubbranchus* based on the branching of the digestive gland and details of their reproductive systems. Until the changes proposed by Martynov (1998b) are critically evaluated by other systematists, we consider it expedient to list our specimens under O'Donoghue's name.

## DISCUSSION

Dall's (1871) report of *Alderia albopapillosa* notwithstanding (see above), no sacoglossans were known from Alaskan waters until Foster (1987a) reported *Hermaea vancouverensis* O'Donoghue, 1924, from Kodiak and Unga Islands in southwest Alaska. Millen (1983) noted this lack of records of sacoglossans from Alaska compared to neighboring regions to the south, and suggested it was due in part to a lack of sampling, as well as to the ease with which these generally small, seasonal herbivores can be overlooked. She predicted that more species would eventually be found in Alaska. Millen (1989) then reported *Aplysiopsis enteromorphae* Cockerell & Eliot, 1905 (as *A. smithi* (Marcus, 1961)) and *Stiliger fuscovittatus* Lance, 1962, from southeast Alaska; and Behrens (1998) reported *Placida dendritica* (Alder & Hancock, 1843) from Chichagof Island, southeast Alaska. Our observations of *Alderia modesta* and *Olea hansineensis* in Prince William Sound bring to six the number of sacoglossan species known from the Aleutian biogeographic province, which extends from the Queen Charlotte Islands out into the Aleutians and into the Bering Sea as far north as Nunivak Island (Briggs, 1974). All six of these species are also known from British Columbia and California (Millen, 1980; Behrens, 1991), leaving only two species from the Oregonian province, *Elysia hedgpethi* Marcus, 1961, and *Aplysiopsis oliviae* (MacFarland, 1966), yet to be found in Alaskan waters (Behrens, 1991; Trowbridge, 2002). The sacoglossan fauna of the Gulf of Alaska is therefore very similar to that of the neighboring and more extensively studied Oregonian Province, but is probably more seasonal in occurrence owing to the more extreme winter conditions in the former. No sacoglossans are known yet from the Aleutian Islands and Bering Sea,

and we did not find any sacoglossans during surveys of Kachemak Bay, off of the lower Cook Inlet, conducted during July 2000.

Three of the opisthobranch species whose ranges we extend (*Alderia modesta*, *Palio dubia*, and *Cuthona pushtulata*) are also known from the north Atlantic and are therefore circumboreal in distribution (Thompson, 1976; Thompson & Brown, 1984). The same may also apply to *Ancula pacifica* and *Eubbranchus olivaceus*, depending on their above-mentioned taxonomic relationships to the north Atlantic *Ancula gibbosa* and *Eubbranchus rupium*, respectively. An additional four species (*Adalaria jannae*, *Diaulula sandiegensis*, *Palio dubia*, and *Triopha catalinae*) are known from either the Sea of Japan or northwest Pacific Ocean and therefore have amphi-Pacific distributions (Behrens, 1991; Martynov, 1998a, personal communication to JHRG 17 February 2001). The same may apply to *Eubbranchus olivaceus* and *Alderia modesta*, depending on their respective relationships to *Eubbranchus rupium* and *Alderia* sp. reported from the northwestern Pacific by Martynov (1998a, b). One species, *Calycidoris guentheri*, is strictly arctic in distribution (see Platts, 1985; Lee & Foster, 1985), and the remaining species (*Olea hansineensis*, *Adalaria* sp. 1, *Archidoris odhneri*, *Doridella steinbergae*, *Geitodoris heathi*, *Armina californica*, *Janolus fuscus*, *Cuthona albocrusta*) are found only in the northeastern Pacific (Behrens, 1991). The proportion of species with these different distributions reflect those for the Alaska opisthobranch fauna as a whole (see Lee & Foster, 1985).

Most of the range extensions documented above are for species that are either: (1) easily overlooked (owing to their small size, cryptic coloration, or seasonal occurrence), (2) recorded for the first time from remote, little-studied parts of Alaska, or (3) already known from both the northeastern Pacific Ocean and either the north Atlantic Ocean or the northwest Pacific Ocean. Therefore, we consider most, if not all, of these range extensions to be the result of increased or fortuitous search efforts, rather than actual range expansions by the species themselves. One possible exception to this may be represented by *Janolus fuscus*, a conspicuous arminacean that commonly reaches 30 to 40 mm in length (personal observations). Extensive faunal surveys conducted along the entire coast of British Columbia in the 1950s and 1960s found this species only as far north as central Vancouver Island (Bernard, 1970). Lambert (1976) and Robilliard & Barr (1978) then extended the range of *J. fuscus* to sites in southeast Alaska. While these and our own records are consistent with a recent range expansion by this species, we cannot rule out that *J. fuscus* has been a rare or intermittent member of the Alaskan fauna for a much longer time period.

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## Mollusca of Assateague Island, Maryland and Virginia: Additions to the Fauna, Range Extensions, and Gigantism

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**Abstract.** Collections of 108 species of marine and estuarine mollusks from and around Assateague Island, Maryland and Virginia, from 1991 to 1996, vary from and extend the known species lists generated by three previously published collections over the past 100 years. Extensive sampling, including benthic grabs, trawls, and hand collecting, has added 54 species of mollusks (20 bivalves, 31 gastropods, one polyplacophoran, and two cephalopods) to the 1914 list of Henderson & Bartsch and 46 (19 bivalves, 26 gastropods and one cephalopod) to that of Counts & Bashore from 1991. Homer et al. in 1997 provided a mollusk survey of Maryland coast bays and listed 73 molluscan species (including 10 species recorded as shells only and eight as taxonomic uncertainties). To the latter we have added 51 molluscan taxa they did not find (19 bivalves, 29 gastropods, one polyplacophoran, and two cephalopods). All collections represent a total described malacofauna of this region of 146 shallow-water species excluding undescribed or non-described taxa in earlier papers. Within the populations of some of the species collected were a few exceptionally large individuals, adding to previous records of unusually large specimens of mollusks from this region of the Atlantic coast. Additionally, some species of mollusks (*Tectura testudinalis*, *Eupleura semisulcata* [Gastropoda], *Tridonta borealis* [Bivalvia]) and some non-mollusks (the ascidian *Ecteinascidia turbinata* and a confirmation of an extension of the anthozoan *Peachia parasitica*) have been found in the waters surrounding Assateague, well outside of their previously reported geographic ranges. The results of the present study suggest the need for a re-evaluation of possible environmental shifts that could have taken place since the collections of the early 1900s and have elsewhere been implicated in the change of malacofauna of Assateague Island since that time. Additionally, range extensions reported could reflect a subtle geographic transition zone, newly introduced species, or, most likely, an understudied coastal area.

### INTRODUCTION

Three previous notable surveys of marine and estuarine mollusks have been conducted at or just adjacent to Assateague Island along the Maryland and Virginia, USA, coast. The first, by Henderson & Bartsch (1914), reported 37 species of bivalves and 44 species of gastropods from nearby Chincoteague Island, Virginia, from collections made during the course of a week in the summer of 1913. Fourteen of the gastropods reported in their study were described as new species. In particular, among other gastropods, they described as new some very small snails including: *Bittium alternatum virginicum*, *Odostomia pocahontasae*, *O. virginica*, *Turbonilla pocahontasae*, *T. powhatani*, *T. toyatani*, and *T. virginica*. Among the 14 new species were three others they believed were new but of which the specimens were “too poor to serve for description” (Henderson & Bartsch, 1914). It is unlikely that these latter specimens truly represent new species. Within the individual genera, their other “new species” are often difficult to distinguish as morphologically unique, and some are likely subtaxa or ecophenotypes of other species, e.g., *Diastoma virginica* Henderson &

Bartsch 1914 = *Bittium alternatum virginicum* = probably a variant of *Bittium varium* (Pfeiffer, 1840). The validity of several of their new species awaits detailed examination, as many other species described as new by Bartsch have already been placed in synonymy of previously described taxa. Counts & Bashore (1991) made similar collections between April 1988 and August 1989, but expanded their geographic coverage to include all of Assateague Island. They found 73 species of mollusks, 32 species of bivalves, 39 species of gastropods, and one species each of Polyplacophora and Cephalopoda. However, of the 81 valid or newly described species of Mollusca reported by Henderson & Bartsch, only 50 were reported as still present 75 years after their 1913 collection, and Counts & Bashore (1991) reported an additional 25 species not found during the study of Henderson & Bartsch. More recently, Homer et al. (1997) surveyed the mollusks of the Maryland coast in a “shellfish inventory” for the Maryland Department of Natural Resources. The latter study was intended to form a baseline for “future management needs” of the Maryland coast, in particular for commercially important mollusks (e.g., *Crassostrea virginica*, *Mercenaria mercenaria*) of the region. They

recovered 63 live molluscan taxa during their study plus 10 species represented by shells only. Of their recovered species, 16 were previously unrecorded from the Maryland coast.

During several collections from 1994–1996, we found live representatives of 101 species and valves of an additional seven species of mollusks from along areas comparable to these other collections. Our data showed significant variation in the malacofauna reported in all previous studies plus some interesting range extensions, and evidence of “gigantism” among some of the mollusks in the area. This study collected 27 species of mollusks not recorded in the previous three major studies. Similarly, each of the studies had at least some species not found by the others. The faunal variations found among the various studies are significant, and while our overall collection most closely overlaps with that of Counts & Bashore (1991) (in terms of most species matches), interesting differences appear between various collections of gastropods and between all pairs of previous collections. If nothing else, it is clear this mid-Atlantic coastal region has a wide array of microhabitats that hold many hitherto unrecorded taxa.

## METHODS

Quantitative and qualitative sampling was carried out during midsummer, late autumn, and early spring during 1994, 1995, and 1996. All primary shallow-water marine habitats along coastal Assateague and Chincoteague were sampled. Qualitative samples were taken at irregular sites along Assateague, Maryland, and Virginia (Figure 1) with kicknets, Yabby pumps, trawls, seines, and by hand collecting. Habitats sampled qualitatively included jetties, extensive mudflats (Tom’s Cove, Little Tom’s Cove, and Wash Flats), benthic trawls (especially in Cockle and Mosquito creeks), grabs to depths of 15 m (especially near the mouth of Chincoteague Bay at Turner’s Lump and adjacent waters), and oyster beds. The rock jetty at Memorial Park, Chincoteague was also carefully searched for epifauna and crevice dwellers. Since the time of collection, the original rock jetty at Memorial Park has been replaced with a much more extensive wooden (treated) and rock structure and boat launch. Additionally, we sampled the eelgrass beds adjacent to nearby Greenbackville, Virginia.

As part of a larger survey of macroinvertebrates of Assateague Island (Counts & Prezant, 2001), sampling stations were established along transects at uniform distances from shore and/or water depths along the island to include ocean near-shore sandy bottom, bay sandy bottom, bay submerged aquatic seagrass beds, bay intertidal mudflats, fringing marshes, and bay muddy bottom/tidal gut/embalements. Specifically (Figure 1), along each of four separate oceanside transects (O-2, O-7, O-12, O-16), three sampling stations were established at mid-swash

zones, 5 m from shore (subtidal), and 25 m from shore (also subtidal). Twelve transects within Chincoteague Bay were established (B-1 through B-4 and B-7 through B-16), each with four sampling stations that included: mid-swash zone, 0.5 m depth relative to mean high tide (subtidal), 1.0 m depth (subtidal), and 1.5 m depth (subtidal). Six replicates were taken at each site with a small box core sampler. These individual sites are described in the next section.

All samples were preserved with 5% (CaCO<sub>3</sub>) buffered formalin, washed in water and transferred to 70% ethanol for storage. Identifications were made in the laboratory using standard reference works. Collections have been deposited in the mollusk collections of Montclair State University, the University of Maryland Eastern Shore, and the American Museum of Natural History, New York.

Our qualitative data allowed a comparison with the few more complete compilations of molluscan taxa collected from the Assateague and Chincoteague coasts. We used a Bray-Curtis similarity index using PRIMER version 5.0 (Plymouth Routines in Multivariate Ecological Research, Carr [1997]) to compare our species list with those compiled by Henderson & Bartsch (1914), Counts & Bashore (1991), and Homer et al. (1997). Additionally, we used this program to perform cluster analyses among the various studies to find highest levels of similarity in collections. In all analytical work, we discounted any taxa not fully identified in previously published work (e.g., *Turbonilla* sp.).

## RESULTS AND DISCUSSION

### Description of the Study Area

Assateague Island is a barrier island system located on the southern Atlantic coast of Maryland extending southward to the northern coast of Virginia (Figure 1). The island is approximately 58 km in length and averages 0.8 km in width. It is bounded on the north by Ocean City Inlet (separating Assateague from Fenwick Island), on the south by Chincoteague Inlet, on the east by the Atlantic Ocean, and on the west by Sinepuxent and Chincoteague Bays. The average depth of Sinepuxent Bay ranges from 1.0 to 1.5 m, with a 2 m deep channel, and deepens to 5–6 m at Ocean City Inlet. The maximum width of Chincoteague Bay is 11.6 km, and the entire back bay system has an area of 428.9 km<sup>2</sup> (Biggs, 1970). The depth of Chincoteague Bay ranges from 1 to 3 m, deepening to 38 m at Chincoteague Inlet. The southern end of the island contains Tom’s Cove, formed by an eastward-bending sand spit (Fishing Point) and the main body of the island. The average depth of Tom’s Cove is 1 m.

Seiling (1954) described the physical characteristics of the waters surrounding Assateague Island. In summer months, water temperatures are cooler at the inlets and warmer in the shallow bays. In the winter, the pattern is

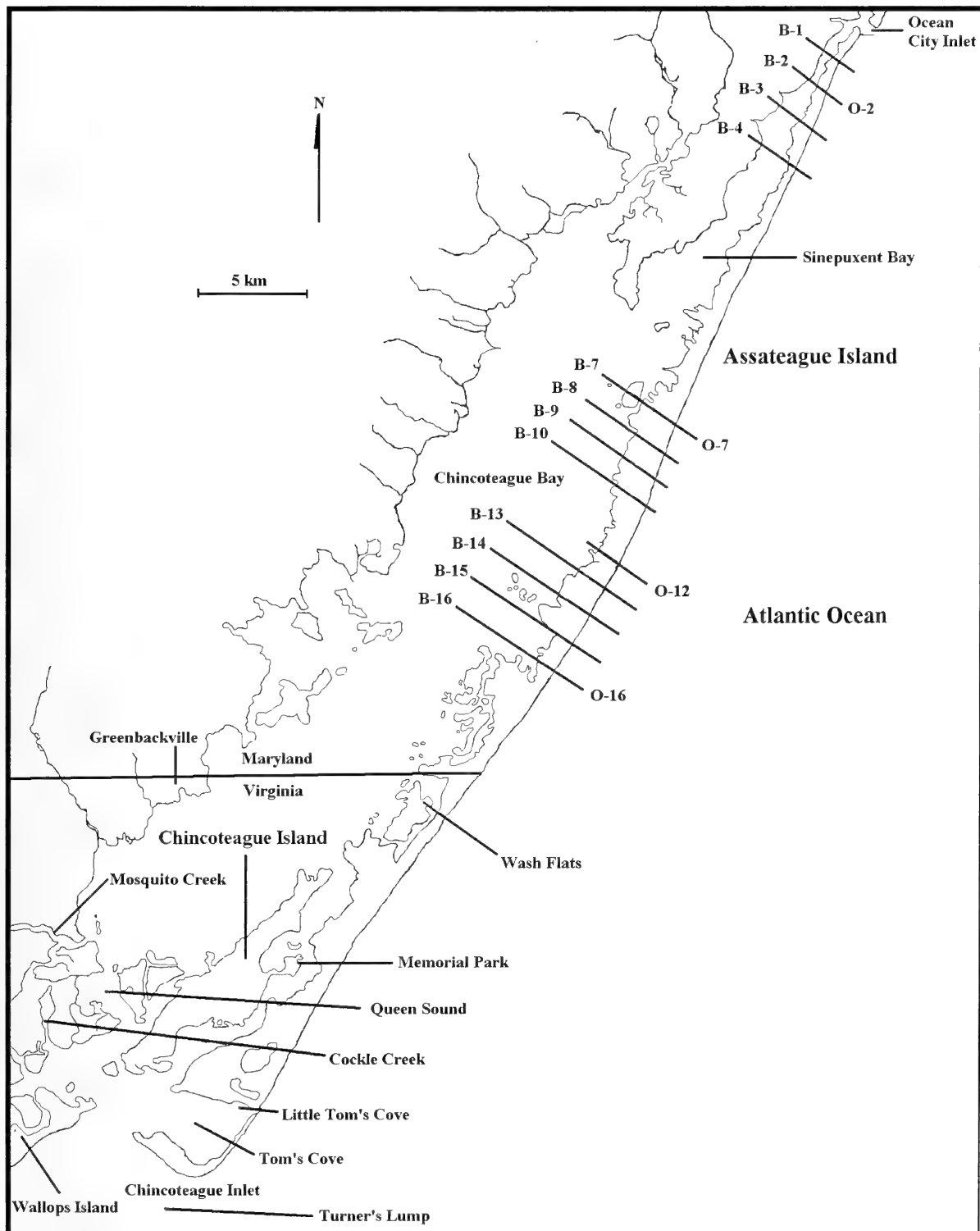


Figure 1. Assateague Island, Maryland and Virginia. The map shows transect lines along Chincoteague Bay (represented by B-transect lines) and ocean coast (represented by O-transect lines). See text for description of transect sites. Other sampling areas are labeled by name.

reversed, and occasionally the bays will freeze over. In summer, salinities decrease toward the inlets where tidal surge mixes seawater with high salinity bay water. The salinity pattern reverses during the winter and spring months. Summer salinity patterns result from a net loss from evaporation that is made up by tidal inflow and minimal freshwater inflow streams on the mainland (Pellenbarg & Biggs, 1970). Summer 1989 was characterized by higher than usual rainfall, and salinities ranged from 24 to 35 ppt in Chincoteague Bay, the highest salinities being measured at the inlets. Tidal amplitudes are not remarkable, being approximately 1 m at the inlets and 0.33 m in the bays. Tidal currents of Chincoteague and Sinepuxent bays are mostly independent of the non-tidal oceanic currents, and water flows away from the inlets at Ocean City and at Chincoteague as the tide rises (Pellenbarg & Biggs, 1970). Bay water circulation is such that the total water movement of the bays allows a daily water exchange of approximately 7.5% from outside sources (Pritchard, 1960). Pellenbarg & Biggs (1970) reported the bays to be essentially stagnant and intensely heated and stratified during the summer months. Seiling (1954) noted that currents throughout the bays, although of no great magnitude, could have some influence on shellfish larval distribution.

Atlantic coastal waters of Assateague Island are shallow, and Pellenbarg & Biggs (1970) noted that they become rapidly stratified by mid-April and that there is little mixing between thermally stratified waters. Summer surface currents are generally onshore, and the entire water mass has a northerly drift, perhaps due to the nearby Gulf Stream (Pellenbarg & Biggs, 1970).

While the overall exposed beach along Assateague was quite uniform (mid-energy medium coarse sand sediment), the bay side was somewhat variable. The sites used for transects (as indicated on Figure 1) include the following (B = Bay side; O = Ocean side):

**B-1:** 1 km south of Ocean City Inlet. A sandy shore bordering a *Spartina alterniflora* dominant marsh. Relatively firm substratum with some fragmented macroalgae accumulations. The 1.0 m depth site along the transect was located 40 m from shore indicating a relatively shallow beach slope. Sediments from deeper (0.5 and 1.0 m depths) sites were muddy with a diatom or cyanobacter coating (slippery surface over firm mud). Sediments from all depths had a hydrogen sulfide odor, which was strongest at the 0.5 m depth site.

**B-2:** 3 km south of Ocean City Inlet. The swash zone occurred as an overwash flat with soft sediments; sporadic algal clumps; swash zone sediment was dark colored with hydrogen sulfide odor; 0.5 m depth subtidal sediments had a muddy silt covering. The gently sloping beach dropped to the 1.0 m depth site at 50 m from the swash zone.

**B-3:** 5 km south of Ocean City Inlet. Swash zone is an eroding salt marsh perimeter. Substratum in swash had

a hydrogen sulfide odor; no odor from subtidal sediments; No shell fragments. 1.0 m depth site located 50 m from shore.

**B-4:** 7 km south of Ocean City Inlet. Very shallow decline to about 0.75 m. Sandy sediments. No sulfide odors in sediments collected. 1.0 m depth station located 80–85 m from shore.

**B-7:** 13 km south of Ocean City Inlet. Swash zone along a *Spartina* marsh gut, other station sites within gut. Turbid water caused by suspended solids; sediment anaerobic close to surface. Steeper slope beach with 1.0 m depth located 10 m from shore. Some submerged vegetation at 1.0 m depth.

**B-8:** 15 km south of Ocean City Inlet. Very shallow sloping beach with swash zone within *Spartina* marsh and 0.5 and 1.0 m depth stations in embayment. 1.0 m depth station located 125 m from shore. Sandy, firm substratum; anaerobic in shallower stations.

**B-9:** 17 km south of Ocean City Inlet. Beach front a bit steeper with 1.0 m meter station located 30 m from shore. Swash zone at edge of shallow gut with 0.5 m and 1.0 m stations located within submerged aquatic vegetation (SAV). Soft sediments black to gray in color.

**B-10:** 19 km south of Ocean City Inlet. Swash zone along marsh front with deeper stations in shallow gut about 45 m from shore. Plant fragments in swash zone; swash zone sediments with hydrogen sulfide odor.

**B-13:** 25 km south of Ocean City Inlet. Very shallow beach with 1.0 m depth located 140 m beyond swash zone. Entire station part of a tidal flat with fine sand substratum; only swash zone sediment had a sulfide odor.

**B-14:** 27 km south of Ocean City Inlet. 1.0 m depth located only 15 m from shore, comparatively steep beach. Swash zone an eroding marsh front; 1.0 m depth station with SAV (*Zostera marina*). Swash zone sediment clumped mud grading to fine to medium sands with increasing depth.

**B-15:** 29 km south of Ocean City Inlet. Relatively steep beach with 1.0 m depth located 20 m from shore. Swash zone part of *Spartina* marsh; 1.0 m depth with SAV. Firm substratum with sulfide odor in swash zone sediments only.

**B-16:** 31 km south of Ocean City Inlet. Relatively steep beach with 1.0 m depth located 20 m from shore. Swash zone is part of Marsh Island Cove, a low *Spartina* marsh. Eelgrass beds at 0.5 and 1.0 m depths. Swash zone sediments with sulfide odor. Sediments in swash zone muddy with probable cyanobacter and/or diatom cover. Deeper sites with sandier substratum.

Ocean sites were located in direct line with bay sites B-2, 7, 12, and 17 and were nearly identical in general appearance: fine to medium sand, low to mid-energy beaches with mid-grade slope. Each ocean transect had samples taken (six replicates) at the swash zone, 0.5 and 1.0 m depths.

## Malacofauna, Environment, and Changes through Time and among Studies

Assateague and Chincoteague Islands and their near-shore environments offer a wide array of soft sediment habitats ranging from mud flats to marshes, sea grass beds to sand beaches. Numerous jetties and piers add artificial hard substrata that are densely colonized by epifauna. Oyster beds, natural and planted, offer an additional hard surface and crevice habitat for various mollusks. A large number of variably detailed general surveys have included at least part of our study sites. Casey & Wesche (1981) examined the coastal benthos of Maryland's bays. Their seasonal collections included two locations in Chincoteague Bay. Using an otter trawl (6.35 mm mesh) and a Ponar grab (sieved at 1.0 mm), they recovered a total of 15 species of mollusks. They also collected another 142 species of non-molluscan benthic organisms. In all of their samples, *Mytilus edulis* dominated in terms of sheer numbers, composing 87% of all individuals collected ( $T = 50,033$  in spring and winter samples). The bias toward *M. edulis* probably indicates a bias in sample sites and thus sampling substratum and habitat. Blue mussels are frequently not only dominant organisms in terms of sheer numbers in a community, but also can serve to inhibit settlement of other species, thus reducing overall diversity. Seasonally, however, the authors found a significant overall decline in the number of organisms and number of taxa recovered from their spring sampling period (late April to early May) to their summer sampling (late July to early August). In spring 1981, they collected 11 species of mollusks. This dropped to nine in the summer collection. In fall 1981, they collected nine species of mollusks (six gastropods, three bivalves) while in their winter collection this dropped to a total of five (two gastropods, three bivalves). The most commonly collected species for all seasons combined was the relatively small *Tellina agilis*, an infaunal bivalve usually inhabiting fine sand to mud. The likelihood that there were only 15 species of mollusks present during the latter study is remote. More likely, the low diversity reflects a combination of compromised sampling techniques (the authors allude to grab samples that lacked adequate "bite"), relatively infrequent sampling, and poor preservation (some specimens were difficult to identify because of preservation problems).

Similar to the study noted above, Drobek et al. (1970), in a final report on the environment of Assateague Island, listed only 12 species of mollusks. These authors sampled 64 sites within Chincoteague Bay, from Ocean City Inlet to the Virginia border, using a shallow-water escalator harvester. They note that this "gear permits a quantitative removal from the bottom of all bottom-dwelling animals over approximately 1 cm in length." Thus, their sampling missed the smaller biota.

More comprehensive studies targeted the malacofauna

specifically and revealed a much more diverse molluscan biota. Henderson & Bartsch (1914) reported 81 species (excluding two *Turbonilla* that they presumed new but did not describe) from Chincoteague Island. Counts & Bashore (1991) found 73. (Note: The text and tables in Counts & Bashore [1991] are not in agreement; the appropriate counts for that paper are taken from their Table 1.) Homer et al. (1997) reported a total of 73 molluscan taxa from the Maryland coast. We found 108 species of mollusks from this region (Table 1), a total greater than that in any previous study. In all studies combined, there are 146 species of mollusks listed from this region (also excluding undescribed or nondescribed taxa listed by Homer et al. [1997]). Homer et al. (1997) suggested that there were several factors that could be associated with the molluscan diversity found. These include the polyhaline environment that "allows the more tolerant marine species to exploit this system, adding to the true estuarine species." Additionally, they note the diversity of benthic habitats based on a wide array of sediment types as a possible factor accounting for the relatively high molluscan diversity. In our collections, mollusks were found in a wide array of habitats that reflect the diversity of substrata and other resources available in the region for initial settlement (see Table 2 for listing of general habitat distribution and specific localities based on transects). Lastly, Homer et al. (1997) suggested that the location of Chincoteague Bay offers a transitional zone, located at the south end of the Virginian province, allowing a blending with several Carolinian species. Nevertheless, among all studies through time, we see significant differences among total species listed.

We found 47 species of bivalves, compared to 32 by Counts & Bashore (1991), 37 by Henderson & Bartsch (1914), and 31 by Homer et al. (1997) (Table 3). Of these, we found 19 not reported by Counts & Bashore (1991), 20 not found by Henderson & Bartsch (1914), and 19 not reported by Homer et al. (1997) (Table 4). On the other hand, Counts & Bashore reported six bivalve species we did not discover, Henderson & Bartsch found 10 not on our present list, and Homer et al. (1997) reported three that we did not recover. These kinds of differences are evaluated more carefully below where we examine specific similarities and differences in malacofauna. In some cases they represent subspecies of questionable validity; in others, they could represent drift of empty valves (reported as such in our study but not differentiated from living mollusks by Henderson & Bartsch (1914) and Counts & Bashore (1991). In all, the three earlier studies and the present study have a total overlap of only 13 species of bivalves. We found nine species of bivalves not found by Henderson & Bartsch (1914), Counts & Bashore (1991), nor Homer et al. (1997). Thus only about 22% of the species of bivalves we found in the present study were found in all three previous studies.

Of the 58 reported gastropods in the present study, we

Table 1

Mollusca of Assateague Island, Maryland and Virginia. A comparison of results from Henderson & Bartsch (1914) (A), Counts & Bashore (1991) (B), Homer et al. [coastal Maryland study, 1993–1996] (1997) (C) and the present study (D). Notes are presented in right hand column. + = Present; – = Absent; **G** = “Giant” specimen(s); **R** = Range extension; **S** = Shell only. (Note: Counts Bashore [1991] did not distinguish live animals from shells only.) In cases where the taxonomic validity of a particular species is in question (either because of a debate or question in the literature; overviews in Turgeon et al., 1998), it is also indicated under the notes column. Undescribed species, species thought to be new, or nondescribed taxa (e.g., two species of *Turbonilla* in Henderson & Bartsch and seven species of gastropods in Homer et al. listed as sp.) are not included in this list nor in any numerical analyses.

Species	A	B	C	D	Notes
<b>BIVALVIA</b>					
<i>Abra aequalis</i> (Say, 1822)	+	–	–	–	
<i>Aligena elevata</i> (Stimpson, 1851)	–	–	+	+	
<i>Anadara ovalis</i> (Bruguière, 1789)	+	+	+	+	<i>Scapharca campechiensis pexata</i> in Henderson & Bartsch (1914)
<i>Anadara transversa</i> (Say, 1822)	+	+	+	+	<i>Scapharca transversa</i> Say in Henderson & Bartsch (1914)
<i>Anomia simplex</i> d'Orbigny, 1842	+	+	+	+	<i>Anomia glabra</i> also listed by Henderson & Bartsch (1914) but almost certainly an error
<i>Argopecten gibbus</i> (Linnaeus, 1758)	+	+	–	–	Henderson & Bartsch (1914) list as <i>Pecten gibbus irradians</i> —probably a juvenile <i>A. irradians irradians</i>
<i>Argopecten irradians</i> f. <i>concentricus</i> (Say, 1822)	–	+	–	–	Planted by M. Castagna, VIMS, Wachapreague, VA
<i>Argopecten irradians irradians</i> (Lamarck, 1819)	–	+	S	S	Planted by M. Castagna, VIMS, Wachapreague, VA
<i>Astarte castanea</i> (Say, 1822)	+	+	–	–	
<i>Barnea truncata</i> (Say, 1822)	–	+	–	+	
<i>Brachidontes exustus</i> (Linnaeus, 1758)	–	–	–	+	
<i>Chione cancellata</i> (Linnaeus, 1767)	+	+	–	S	
<i>Circomphalus strigillinus</i> (Dall, 1902)	–	–	–	+	
<i>Cyrenoidea floridana</i> (Dall, 1896)	–	–	–	+	
<i>Corbula contracta</i> Say, 1822	+	–	–	+	
<i>Crassinella lunulata</i> (Conrad, 1834)	+	+	–	–	
<i>Crassostrea virginica</i> (Gmelin, 1791)	+	+	+	+	
<i>Cyclinella tenuis</i> (Récluz, 1852)	–	–	R	–	
<i>Cyclocardia borealis</i> (Conrad, 1831)	+	+	–	–	<i>Venericardia granulosa</i> Say = <i>Cardita borealis</i> Henderson & Bartsch in Henderson & Bartsch 1914)
<i>Cyrtopleura costata</i> (Linnaeus, 1758)	+	+	+	+	
<i>Dinocardium robustum</i> (Lightfoot, 1786)	S	–	–	–	
<i>Divaricella quadrisulcata</i> (d'Orbigny, 1842)	+	+	–	S	
<i>Donax variabilis</i> Say, 1822	+	+	+	+	
<i>Ensis directus</i> Conrad, 1843	–	+	+	+	
<i>Ensis minor</i> Dall, 1900	+	–	–	–	
<i>Gemma gemma</i> (Totten, 1834)	–	+	+	+	Very common on mudflats, within <i>Limulus</i> depressions
<i>Geukensia demissa</i> (Dillwyn, 1817)	–	+	+	+	
<i>Gouldia cerina</i> (C.B. Adams, 1845)	–	–	–	+	
<i>Ischadium recurvum</i> (Rafinesque, 1820)	–	+	+	–	
<i>Laevicardium mortonii</i> (Conrad, 1830)	+	–	–	–	
<i>Linga pensylvanica</i> (Linnaeus, 1758)	S	–	–	–	<i>Phacoides aurantia</i> Deshayes in Henderson & Bartsch (1914)
<i>Lyonsia hyalina</i> Conrad, 1831	+	–	+	G	Rare intertidally
<i>Macoma balthica</i> (Linnaeus, 1758)	–	+	+	+	
<i>Macoma tenta</i> (Say, 1834)	+	–	+	+	<i>Psammacoma tenta</i> Say in Henderson & Bartsch (1914)



Table 1  
Continued.

Species	A	B	C	D	Notes
<i>Mercenaria mercenaria</i> (Linnaeus, 1758)	+	+	+	+	
<i>Mulinia lateralis</i> (Say, 1822)	+	+	+	+	
<i>Mya arenaria</i> Linnaeus, 1758	+	+	S	+	
<i>Mysella planulata</i> (Stimpson, 1851)	—	—	+	—	
<i>Mytilus edulis</i> Linnaeus, 1758	+	+	+	+	
<i>Noetia ponderosa</i> (Say, 1822)	+	+	+	+	
<i>Nucula proxima</i> Say, 1822	+	—	+	+	Subtidal only at Turner's Lump
<i>Nuculana acuta</i> (Conrad, 1831)	+	—	—	+	
<i>Petricola pholadiformis</i> (Lamarck, 1818)	+	+	+	+	
<i>Pitar morrhuanus</i> (Linsley, 1848)	+	—	+	+	
<i>Placopecten magellanicus</i> (Gmelin, 1791)	—	—	—	S	
<i>Pleuromeris tridentata</i> (Say, 1826)	+	—	—	R	
<i>Polymesoda caroliniana</i> Bosc, 1802	—	—	—	+	
<i>Raeta plicatella</i> (Lamarck, 1818)	+	+	—	+	
<i>Siliqua costata</i> Say, 1822	—	—	—	S	
<i>Solemya velum</i> Say, 1822	—	+	+	+	Very common near Wash Flats, approx. 1–1.5 m depth
<i>Solen viridis</i> Say, 1821	—	+	+	+	
<i>Spisula solidissima</i> (Dillwyn, 1817)	+	+	+	+	
<i>Spisula solidissima similis</i> (Say, 1822)	+	—	—	—	Controversial subspecies, see Cargnelli et al. (1999)
<i>Tagelus divisus</i> (Spengler, 1794)	+	—	+	+	
<i>Tagelus plebius</i> (Lightfoot, 1786)	+	+	+	+	<i>Tagelus gibbus</i> Spengler in Henderson & Bartsch (1914)
<i>Tellina aequistriata</i> Say, 1824	—	—	—	+	
<i>Tellina agilis</i> Stimpson, 1857	+	+	+	+	<i>Angulus tenera</i> Say in Henderson & Bartsch (1914)
<i>Tellina versicolor</i> DeKay, 1843	—	—	—	+	
<i>Teredo navalis</i> Linnaeus, 1758	—	—	—	+	In wood debris on beaches
<i>Tridonta borealis</i> (Schumacher, 1817)	—	—	—	R	Subtidal only at Turner's Lump; Formerly <i>Astarte borealis</i>
<i>Yoldia limatula</i> (Say, 1831)	+	—	—	+	Subtidal only at Turner's Lump
<b>GASTROPODA</b>					
<i>Acanthodoris pilosa</i> (Müller, 1776)	—	+	—	+	
<i>Acteocina bidentata</i> (d'Orbigny, 1841)	+	+	+	+	In Henderson & Bartsch (1914) as <i>Cylichnella biplicata</i> & Homer et al. as <i>C. bidentata</i>
<i>Acteocina canaliculata</i> (Say, 1822)	+	+	+	+	<i>Tornatina canaliculata</i> Say in Henderson & Bartsch (1914)
<i>Acteon punctostriatus</i> Adams, 1840	—	—	—	+	
<i>Assiminea succinea</i> (Pfeiffer, 1840)	—	—	—	+	
<i>Astyris lunata</i> (Say, 1826)	+	+	+	+	Formerly <i>Mitrella lunata</i>
<i>Bittium alternatum</i> (Say, 1822)	—	—	—	+	<i>Zostera</i> beds; possibly an ecological variant of <i>B. varium</i> . <i>Fissurella alternata</i> Say in Henderson & Bartsch (1914)
<i>Bittium alternatum virginicum</i> (Henderson & Bartsch, 1914)	G	—	—	—	Probably not a valid (sub)taxon but a variant of <i>B. varium</i>
<i>Bittium varium</i> (Pfeiffer, 1840)	—	—	+	+	<i>Zostera</i> beds
<i>Boonea bisuturalis</i> (Say, 1822)	—	—	—	+	On <i>Crassostrea virginica</i>
<i>Boonea impressa</i> (Say, 1822)	+	—	S	+	On <i>Crassostrea virginica</i>
<i>Boonea seminuda</i> (C. B. Adams, 1839)	—	—	—	+	= <i>Odostomia toyatani</i> of Henderson & Bartsch (1914); On <i>Crassostrea virginica</i>
<i>Buccinum undatum</i> Linnaeus, 1758	—	+	—	—	
<i>Busycon carica</i> (Gmelin, 1791)	+	+	—	+	Very large specimen. <i>Fulgur carica</i> in Henderson & Bartsch (1914)

Table 1  
Continued.

Species	A	B	C	D	Notes
<i>Busycon sinistrum</i> (Hollister, 1958)	+	+	—	—	<i>Fulgar perversa</i> Linnaeus in Henderson & Bartsch (1914)
<i>Busycotypus canaliculatus</i> (Linnaeus, 1758)	+	+	+	+	<i>Sycotypus canaliculatus</i> Say in Henderson & Bartsch (1914)
<i>Cerithidea scalariformis</i> (Say, 1826)	—	—	—	+	
<i>Cerithiopsis emersoni</i> (C.B. Adams, 1839)	—	—	S	—	
<i>Cerithiopsis greenii</i> (C.B. Adams, 1839)	+	—	+	+	
<i>Clathurella jewetti</i> (Stearns, 1873)	+	—	—	—	Uncertain taxon
<i>Conchiolepis parasitica</i> Stimpson, 1858	—	—	—	+	
<i>Costoanachis avara</i> (Say, 1822)	+	+	+	+	<i>Anachis avara</i> in other reports
<i>Cratena pilata</i> (Gould, 1870)	—	+	—	—	
<i>Crepidula convexa</i> Say, 1822	+	+	+	G	
<i>Crepidula fornicata</i> (Linnaeus, 1758)	+	+	+	+	Very large specimens
<i>Crepidula plana</i> (Say, 1822)	+	+	+	+	
<i>Cresis virgula</i> (Rang, 1828)	—	+	—	—	
<i>Crucibulum striatum</i> Say, 1824	—	+	—	S	
<i>Diodora cayenensis</i> (Lamarck, 1822)	+	+	S	S	
<i>Doris verrucosa</i> Linnaeus, 1758	—	—	+	—	Chincoteague only
<i>Elysia chloritica</i> (Gould, 1870)	—	—	—	+	<i>Zostera</i> beds
<i>Epitonium angulatum</i> (Say, 1830)	—	+	—	—	
<i>Epitonium humphreysi</i> (Kiener, 1838)	+	+	—	+	<i>E. sayana</i> Dall in Henderson & Bartsch (1914)
<i>Epitonium multistriatum</i> (Say, 1826)	+	+	+	+	
<i>Epitonium rupicolum</i> (Kurtz, 1860)	+	+	+	+	<i>E. lineata</i> Say in Henderson & Bartsch (1914)
<i>Eptinioum virginicum</i> (Henderson & Bartsch, 1914)	+	—	—	—	Uncertain taxonomic validity
<i>Eupleura caudata</i> (Say, 1922)	+	+	+	+	
<i>Eupleura sulcidentata</i> Dall, 1890	—	—	—	R	Possible imports with oysters
<i>Haminoea solitaria</i> (Say, 1822)	—	—	+	+	
<i>Hydrobia totteni</i> Morrison, 1954	—	—	—	+	
<i>Inodrillia dalli</i> (Verrill & Smith, 1882)	—	+	—	+	
<i>Kurtziella cerina</i> (Kurtz & Stimpson, 1851)	+	+	—	+	<i>Mangilia cerina</i> Kurtz & Stimpson in Henderson & Bartsch (1914)
<i>Kurtziella limonitella</i> (Dall, 1884)	—	—	—	R	
<i>Littoraria irrorata</i> Say, 1822	+	+	—	+	
<i>Littorina littorea</i> (Linnaeus, 1758)	—	+	—	+	
<i>Littorina saxatilis</i> (Olivi, 1792)	—	+	—	+	
<i>Lunatia heros</i> (Say, 1822)	+	+	—	—	
<i>Lunatia pallida</i> (Broderip & Sowerby, 1829)	+	—	—	—	
<i>Lunatia triserata</i> (Broderip & Sowerby, 1829)	—	+	—	—	
<i>Marginella roscida</i> Redfield, 1860	+	—	—	—	<i>Marginella apicina borealis</i> Verrill in Henderson & Bartsch (1914)
<i>Melampus bidentatus</i> Say, 1922	—	+	+	+	
<i>Melanella intermedia</i> (Cantraine, 1835)	+	+	+	+	Usually found on sea cucumber <i>Holothuria impatiens</i> (Abbott, 1974); Henderson & Bartsch (1914) list <i>M. oleacea</i> —almost certainly <i>M. intermedia</i>
<i>Nassarius obsoletus</i> (Say, 1822)	+	+	+	+	
<i>Nassarius trivittatus</i> (Say, 1826)	+	+	+	+	<i>Tritia trivittata</i> Say in Henderson & Bartsch (1914)
<i>Nassarius vibex</i> (Say, 1822)	+	+	+	+	
<i>Natica pusilla</i> Say, 1822	+	—	—	—	Listed by Henderson & Bartsch (1914) but not so indicated in Table 1 in Counts & Bashore (1991)
<i>Neverita duplicatus</i> (Say, 1822)	+	+	+	+	
<i>Odostomia pocahontasae</i> Henderson & Bartsch, 1914	+	—	+	+	Uncertain taxonomic validity; on <i>Crassostrea virginica</i>

Table 1  
Continued.

Species	A	B	C	D	Notes
<i>Odostomia toyatani</i> Henderson & Bartsch, 1914	+	-	-	-	Uncertain taxonomic validity
<i>Odostomia virginica</i> Henderson & Bartsch, 1914	+	-	-	-	
<i>Olivella mutica</i> (Say, 1822)	-	+	-	+	
<i>Ovatella myosotis</i> (Draparnaud, 1801)	-	-	-	+	
<i>Pyramidella candida</i> Mörch, 1875	-	-	-	+	
<i>Pyramidella crenulata</i> (Holmes, 1860)	-	-	R	+	Found in Chincoteague Bay in 1981 by Casey & Wesche (1982)
<i>Pyrgocythara plicosa</i> (C.B. Adams, 1850)	-	-	+	+	
<i>Retusa obtusa</i> (Montagu, 1807)	-	-	-	+	
<i>Sayella fusca</i> (C.B. Adams, 1839)	-	-	-	+	Population with variable eyes; under rocks, Chincoteague
<i>Seila adamsi</i> (H.C. Lea, 1845)	-	-	+	S	
<i>Sinum perspectivum</i> (Say, 1831)	+	+	-	+	<i>Sigaretus perspectivus</i> Say in Henderson & Bartsch (1914)
<i>Stramonita haemastoma floridana</i> (Conrad, 1837)	-	+	S	+	Formerly <i>Thais haemastoma</i>
<i>Tectura testudinalis</i> (Müller, 1776)	-	-	-	R	Very small specimens
<i>Terebra concava</i> Say, 1827	+	-	-	-	
<i>Terebra dislocata</i> (Say, 1822)	+	+	-	+	
<i>Triphora nigrocincta</i> (C.B. Adams, 1839)	+	-	S	-	
<i>Triphora pyrrha</i> Henderson & Bartsch, 1914	+	-	-	-	
<i>Turbonilla interrupta</i> (Totten, 1835)	-	-	+	+	
<i>Turbonilla pocahontasae</i> Henderson & Bartsch, 1914	+	-	S	-	Described by Henderson & Bartsch (1914) in their original collection; questionable taxonomic validity
<i>Turbonilla powhatani</i> Henderson & Bartsch, 1914	+	-	+	-	Described by Henderson & Bartsch (1914) in their original collection; questionable taxonomic validity
<i>Turbonilla toyatani</i> Henderson & Bartsch, 1914	+	-	-	-	Described by Henderson & Bartsch (1914) in their original collection; questionable taxonomic validity
<i>Turbonilla virginica</i> Henderson & Bartsch, 1914	+	-	-	-	Described by Henderson & Bartsch (1914) in their original collection; questionable taxonomic validity
<i>Urosalpinx cinerea</i> (Say, 1822)	G	+	+	+	Very large specimens reported by Baker, 1951, Chincoteague
<b>CEPHALOPODA</b>					
<i>Loligo pealeii</i> Lesueur, 1821	-	+	-	+	
<i>Loliguncula brevis</i> (Blainville, 1823)	-	-	-	+	
<b>POLYPLACOPHORA</b>					
<i>Chaetopleura apiculata</i> (Say, 1830)	-	+	-	G	

matched the list of Henderson & Bartsch (1914) with 27 (Table 5). Our list and that of Counts & Bashore (1991) overlapped with 32 species and there was an overlap of 29 species with Homer et al. (1997). All three lists matched with 22 species. We found 14 species of gastropods not found by Henderson & Bartsch (1914), Counts & Bashore (1991), nor Homer et al. (1997). On the other hand, Henderson & Bartsch (1914) noted 17 species of gastropods that our list lacks; Counts & Bayshore (1991) listed seven not on the current list, and Homer et al. (1997) found five species of gastropods we did not find (Table 4).

Counts & Bashore (1991) suggested that changes in the back-bay circulation and resultant salinity changes could

account for differences in malacofauna over time. This speculation was based, in part, on the work of Castagna & Chanley (1973) who examined the distribution of mollusks along coastal Virginia as influenced by salinity. In addition to other stochastic events that forced opening of new inlets through the island, in 1933 a hurricane opened the Ocean City Inlet, and this new opening was secured by a series of jetties and maintained by dredging. The result of this major inlet was increased flow to, and thus salinity in, the back-bay waters. Specifically, Counts & Bashore (1991) found no pyramidellids, and suggested this was a result of a shift in salinity in the bay due to the inflow of seawater. Pyramidellids were recorded by Henderson & Bartsch (1914) and more recently by Ho-

Table 2

Habitat and local distribution of malacofauna recovered from Assateague and Chincoteague during this study. Habitat distribution is representative and does not indicate total distribution; sites indicated in middle column are from transects described in text. Greenbackville, Virginia is the closest town to a small eelgrass bed; Locations listed as “Assateague Island” represent species common along the long stretch of sand beaches in the state park on Assateague.

Species (Taxon)	Representative collection locations	Habitat note
<b>BIVALVIA</b>		
<i>Aligena elevata</i> (Stimpson, 1851)	Tom's Cove	fine sand-mud; associated with <i>Diopatra</i> tubes
<i>Anadara ovalis</i> (Bruguère, 1789)	Queen Sound attached by byssus to <i>Ulva</i>	muddy sand in shallow water subtidally
<i>Anadara transversa</i> (Say, 1822)	B-16	muddy bottom below low tide line
<i>Anomia simplex</i> d'Orbigny, 1842	B-14, B-16; Cockle Creek	epifaunal on mollusk valves
<i>Argopecten irradians irradians</i> (Lamarck, 1819)	Assateague Island; Greenbackville,	seagrass beds, shallows
<i>Astarte borealis</i> (Schumacher, 1817)	B-3	subtidal, benthic in mud and fine sand
<i>Barnea truncata</i> (Say, 1822)	Wash flats	consolidated mud
<i>Brachidontes exustus</i> (Linnaeus, 1758)	Memorial Park	nestled among rocks
<i>Chione cancellata</i> (Linnaeus, 1767)	Assateague Island	beaches
<i>Circomphalus strigillinus</i> (Dall, 1902)	B-3, B-7, B-8, B-15	medium coarse sand with heavy clam clumps
<i>Cyrenoidea floridana</i> (Dall, 1896)	B-14	brackish water species
<i>Corbula contracta</i> Say, 1822	B-15	medium coarse sand with heavy clay clumps
<i>Crassostrea virginica</i> (Gmelin, 1791)	Memorial Park	oyster beds, pilings, rock jetties
<i>Cyclinella tenuis</i> (Récluz, 1852)	Assateague Island	beaches
<i>Cyrtopleura costata</i> (Linnaeus, 1758)	Greenbackville	firm mud
<i>Deminucula atacellana</i> Schenck, 1939	Turner's Lump; Chincoteague Inlet	fine sand-mud
<i>Divaricella quadrisulcata</i> (d'Orbigny, 1842)	Assateague Island	beaches
<i>Donax variabilis</i> Say, 1822	O-2, O-7, O-12, O-16	high to mid-energy sand beaches
<i>Ensis directus</i> Conrad, 1843	B-4, B-7, B-8; Tom's Cove	low energy embayments in fine to mid sand
<i>Gemma gemma</i> (Totten, 1834)	B-1, B-2, B-3, B-4, B-7, B-8, B-10, B-11, B-13	quiet embayments, mud to fine sand, often in depressions
<i>Geukensia demissa</i> (Dillwyn, 1817)	B-4, B-8, B-14	<i>Spartina</i> marshes, often nested among roots
<i>Gouldia cerina</i> (C.B. Adams, 1845)	Tom's Cove	found attached to loose shell material
<i>Lyonsia hyalina</i> Conrad, 1831	Tom's Cove	less than 2 m depth in fine sand
<i>Macoma balthica</i> (Linnaeus, 1758)	B-14, B-15	common intertidal
<i>Macoma tenta</i> (Say, 1834)	B-2, B-3, B-14	common in shallow sandy water
<i>Mercenaria mercenaria</i> (Linnaeus, 1758)	B-1, B-4, B-8, B-13, B-16	common in shallow sand and mud bays
<i>Mulinia lateralis</i> (Say, 1822)	B-1, B-4, B-7, B-14, B-15; Tom's Cove	shallow sandy quiet waters
<i>Mya arenaria</i> Linnaeus, 1758	Tom's Cove	shallow sandy intertidal to just subtidal
<i>Mytilus edulis</i> Linnaeus, 1758	B-1, B-2, B-8, B-9, B-14	attached to solid objects in marshes
<i>Noetia poderosa</i> (Say, 1822)	Cockle Creek	among shell “litter”
<i>Nucula acuta</i> (Conrad, 1831)	Turner's Lump	fine sand-mud
<i>Nucula proxima</i> Say, 1822	Turner's Lump	fine sand-mud
<i>Petricola pholadiformis</i> (Lamarck, 1818)	Cockle Creek	shell mix
<i>Pitar morrhuanus</i> (Linsley, 1848)	Assateague Island	beaches
<i>Placopecten magellanicus</i> (Gmelin, 1791)	Wash Flats	washed into embayment
<i>Pleuromeris tridentata</i> (Say, 1826)	Cockle Creek	among shell “litter”
<i>Polymesoda caroliniana</i> Bosc, 1802	B-4	sandy-mud
<i>Raeta plicatella</i> (Lamarck, 1818)	Assateague Island	beaches
<i>Siliqua costata</i> Say, 1822	B-4	shallow water sand flats
<i>Solemya velum</i> Say, 1822	B-3, B-7, B-8, B-9, B-10, B-13, B-14, B-15, B-16	shallow water in mud
<i>Solen viridis</i> Say, 1821	B-1; Wallops Beach	shallow water sand flats
<i>Spisula solidissima</i> (Dillwyn, 1817)	O-2, O-12, O-16; Wallops Beach (North)	subtidal on beaches
<i>Tagelus divisus</i> (Spengler, 1794)	B-1, B-8, B-9, B-13, B-14, B-15, B-16, O-16	shallow water
<i>Tagelus plebius</i> (Lightfoot, 1786)	B-4, B-13, B-15, B-16	shallow water in mud-sand intertidal area
<i>Tellina aequistriata</i> Say, 1824	Wash Flats	fine sand/mud
<i>Tellina agilis</i> Stimpson, 1857	B-1, B-2, B-3, B-4, B-8	common in medium to fine sand

Table 2  
Continued.

Species (Taxon)	Representative collection locations	Habitat note
<i>Tellina versicolor</i> DeKay, 1843	B-1, B-2, B-3, B-4, B-7, O-2	common subtidal to 150 ft
<i>Yoldia limatula</i> (Say, 1831)	benthic Turner's Lump	infaunal, soft sediments
<b>GASTROPODA</b>		
<i>Acanthodoris pilosa</i> (Müller, 1776)	Cockle Creek	on sulfur sponges ( <i>Halichondria</i> )
<i>Acteon punctostriatus</i> Adams, 1840	Assateague Island	beach
<i>Acteocina bidentata</i> (d'Orbigny, 1841)	Chincoteague Inlet	fine sand
<i>Acteocina canaliculata</i> (Say, 1822)	B-9, B-15, B-16	estuarine
<i>Assimineia succinea</i> (Pfeiffer, 1840)	Memorial Park	fine sand between rocks
<i>Astyris lunata</i> (Say, 1826)	B-1, B-2, B-3, B-10, B-14, B-15, B-16	found at low tide line
<i>Bittium alternatum</i> (Say, 1822)	B-8, B-9, B-14, B-15; Memorial Park	sand bottoms in quiet water from low tide line
<i>Bittium varium</i> (Pfeiffer, 1840)	B-7, B-8, B-9, B-10, B-13, B-14, B-15, B-16	exceptionally common in eelgrass
<i>Boonea bisuturalis</i> (Say, 1822)	Memorial Park jetty; Queen Sound	on oyster <i>Crassostrea virginica</i>
<i>Boonea impressa</i> (Say, 1822)	Memorial Park jetty	on oyster <i>Crassostrea virginica</i>
<i>Boonea seminuda</i> (C.B. Adams, 1839)	Memorial Park jetty	on oyster <i>Crassostrea virginica</i>
<i>Busycon carica</i> (Gmelin, 1791)	Wallops Beach (North); Assateague Island	beaches
<i>Busycotypus canaliculatus</i> (Linnaeus, 1758)	Assateague Island; Tom's Cove; Wash Flats	beaches
<i>Cerithidea scalariformis</i> (Say, 1826)	B-4	medium sand
<i>Cerithiopsis greenii</i> (C.B. Adams, 1839)	Assateague Island	beaches
<i>Conchiolepis parasitica</i> Stimpson, 1858	Memorial Park	subumbrella Scyphozoan <i>Chrysaora</i>
<i>Costoanachis avara</i> (Say, 1822)	Assateague Island; Tom's Cove	beaches, shallow calm waters
<i>Crepidula convexa</i> Say, 1822	Cockle Creek	on shell rubble
<i>Crepidula fornicata</i> (Linnaeus, 1758)	B-16; Tom's Cove; Wash Flats	attached to hard surfaces, including horse-shoe crabs, shell rubble, etc.
<i>Crepidula plana</i> (Say, 1822)	O-2, O-7, O-12, O-16; Cockle Creek	frequently near internal edge of conch shell inhabited by hermit crab
<i>Crucibulum striatum</i> Say, 1824	Assateague Island	washed up on beach
<i>Diastoma alternatum</i> (Say, 1822)	Greenbackville	seagrass beds
<i>Diodora cayensis</i> (Lamarck, 1822)	Assateague Island	washed up on beaches
<i>Elysia chloritica</i> (Gould, 1870)	Greenbackville	seagrass beds
<i>Epitonium humphreysi</i> (Kiener, 1838)	B-4	fine sand/mud
<i>Epitonium multistriatum</i> (Say, 1826)	Chincoteague Inlet	among coarse sediments and shell rubble
<i>Epitonium rupicolum</i> (Kurtz, 1860)	Assateague Island	beaches
<i>Eupleura caudata</i> (Say, 1922)	Cockle Creek	among shell rubble
<i>Eupleura sulcidentata</i> Dall, 1890	Mosquito Creek; Memorial Park	oyster beds
<i>Haminoea solitaria</i> (Say, 1822)	B-15	white fine sand
<i>Hydrobia totteni</i> Morrison, 1954	B-8, B-9	shallow pools or marshes
<i>Inodrillia dalli</i> (Verrill & Smith, 1882)	Turner's Lump	usually deeper benthos
<i>Kurtziella cerina</i> (Kurtz & Stimpson, 1851)	Greenbackville	seagrass beds
<i>Kurtziella limonitella</i> (Dall, 1884)	Turner's Lump	mud
<i>Littoraria irrorata</i> Say, 1822	B-2, B-3, B-4, B-7, B-8, B-13, B-14	on ( <i>Spartina</i> ) and mud flat surfaces
<i>Littorina littorea</i> (Linnaeus, 1758)	Ocean City Inlet Jetty	cold water species found on rocks or other hard surfaces
<i>Littorina saxatilis</i> (Olivi, 1792)	Memorial Park	rocky surfaces and crevices
<i>Melampus bidentatus</i> Say, 1822	B-15; Chincoteague marsh	intertidal in marshes
<i>Melanella intermedia</i> (Cantraine, 1835)	Chincoteague Inlet	dredged in mixed sediment; shell mix to fine sand
<i>Nassarius obsoletus</i> (Say, 1822)	B-1, B-2, B-3, B-4, B-7, B-8, B-9, B-10, B-13, B-14; Chincoteague Inlet	very common on oozy, warm mud flats
<i>Nassarius trivittatus</i> (Say, 1826)	Turner's Lump	benthic, fine-medium sand
<i>Nassarius vibex</i> (Say, 1822)	B-8; Cockle Creek	common in sand and mudflats
<i>Neverita duplicatus</i> (Say, 1822)	Assateague Island (north end beach)	infaunal predator, just beneath sand surface
<i>Odostomia pocahontasae</i> Henderson & Bartsch, 1914	B-16	fine to very fine sand, some clay present at 1.0 m station
<i>Olivella mutica</i> (Say, 1822)	Assateague Island	beaches
<i>Ovatella myosotis</i> (Draparnaud, 1801)	Memorial Park	in sand between rocks
<i>Pyramidella candida</i> Mörch, 1875	Memorial Park	shallow bays, oyster "parasite"
<i>Pyramidella crenulata</i> (Holmes, 1860)	B-3, B-7, B-8, B-14, B-15, B-16; Chincoteague Bay	shallow bays on sand, mud, or grass

Table 2  
Continued.

Species (Taxon)	Representative collection locations	Habitat note
<i>Pyrgocythara plicosa</i> (C.B. Adams, 1850)	B-16; Memorial Park	fine to very fine sand, some clay present at 1.0 m station
<i>Retusa obtusa</i> (Montagu, 1807)	B-3, B-7, B-9, B-14, B-15, B-16	fine to very fine sand, some clay present at 1.0 m station
<i>Sayella fusca</i> (C.B. Adams, 1839)	B-4, B-7, B-9, B-13; Memorial Park	under rock and shell rubble, intertidal
<i>Seila adamsi</i> (H.C. Lea, 1845)	B-4, B-7, B-9, B-13; Chincoteague Channel	medium to fine sand with very fine sand at 1.5 m station
<i>Sinum perspectivum</i> (Say, 1831)	Assateague Island	mud to fine sand, just subtidal
<i>Stramonita haemastoma floridana</i> (Conrad, 1837)	Memorial Park	oyster and barnacle predator
<i>Tectura testudinalis</i> (Müller, 1776)	B-8, B-14; Cackle Creek	cold water species found on rocks or other hard surfaces
<i>Terebra dislocata</i> (Say, 1822)	O-12	shallow water
<i>Turbonilla interrupta</i> (Totten, 1835)	O-2	shallow water
<i>Urosalpinx cinerea</i> (Say, 1822)	B-16; Indian River Inlet	intertidal to a depth of 25 ft
<b>CEPHALOPODA</b>		
<i>Loligo pealei</i> Lesueur, 1821	Cackle Creek; Walker Point	nektonic
<i>Loliguncula brevis</i> (Blainville, 1823)	Cackle Creek; Turner's Lump	nektonic
<b>POLYPLACOPHORA</b>		
<i>Chaetopleura apiculata</i> (Say, 1830)	Cackle Creek; Queen Sound	benthic on shell rubble

mer et al. (1997). Our study found four species of pyramidellids, three associated with oyster beds. While it is possible that these small gastropods were absent during the 1991 study, it is more likely that they were present and overlooked in the oyster bed refugia. Additionally, a large population of a larger pyramidellid, *Sayella fusca*,

was found under rocks at the Chincoteague Memorial Park jetty in 1994 and 1995. Interestingly, these small gastropods had a large number of eye variations. *Sayella fusca* normally has two small, circular, black basal eyes, but in this population a number of snails had only a left or right eye, sometimes a single central eye, and rarely no eyes. In more recent years, i.e., 1996 and 1997, these pyramidellids were absent at this site, and the original site has now been significantly modified by construction of a pier.

It is relatively easy to account for some of the differences in malacofauna recovered in the four studies. Some small bivalves were likely overlooked previously (e.g., *Aligena elevata*, *Cyrenoida floridana*). Others are probably of very patchy, perhaps rare occurrence (e.g., *Lyonisia hyalina*). It appears that Henderson & Bartsch (1914) did not carefully explore adjacent salt marshes, as indicated by the absence of common marsh fauna (*Geukensia demissa* and *Melampus bidentatus*) from their lists. On the other hand, Henderson & Bartsch (1914) found some larger bivalves not recovered in present collection or in those of Counts & Bashore (1991) (e.g., *Dinocardium robustum*, *Abra aequalis*), and it is more difficult to account for these differences. In some cases, the taxonomy of a group has not been firmly resolved and this could show itself as differences on our respective lists (e.g., pyramidellids, *Bittium-Bittium*, and Turridae, etc.). Additionally, the very small size of many of the gastropods (including species of *Odostomia* and *Turbonilla*) described by Henderson & Bartsch (1914) could lead to misidentifications or severe "splitting" of taxa (perhaps

Table 3

Comparative number of total molluscan species recorded from Assateague and Chincoteague from Henderson & Bartsch (1914), Counts & Bashore (1991), Homer et al. (1997), and the present study. Species identified by Henderson & Bartsch (1914), especially among the very small gastropods, are included in their counts although some remain to be resolved taxonomically. The table also includes taxa identified from valves only (dead shells) as well as taxa that could represent unresolved ecomorphs but are listed in the literature as species. The table does not include the four taxa Henderson & Bartsch list as possible new species or the seven non-described taxa listed by Homer et al.

	Henderson & Bartsch (1914)	Counts & Bashore (1991)	Homer et al. (1997)	Present study
Bivalvia	37	32	31	47
Gastropoda	44	39	34	58
Polyplacophora	0	1	0	1
Cephalopoda	0	1	0	2
Total	81	73	65	108

Table 4

Comparative number of species uniquely found in each study compared to each other study. The number to the left of the diagonal line in each box represents the number of species found in the study listed at the top of the tables versus that on the left. The number to the right of the diagonal line, on the other hand, represents the number of species found uniquely in the study noted on the left of the table versus that on the top. For example, Henderson & Bartsch (1914) found 18 gastropods not found by Counts & Bashore (1991), whereas Counts & Bashore found 13 gastropods not collected by Henderson & Bartsch.

	Henderson & Bartsch		Counts & Bashore		Homer et al.	
	Bivalve	Gastropod	Bivalve	Gastropod	Bivalve	Gastropod
Counts & Bashore	15/10	18/13	xxxx	xxxx	xxxx	xxxx
Homer et al.	17/11	19/10	9/8	19/14	xxxx	xxxx
Present Study	10/20	17/31	9/19	7/26	3/19	5/29

based on ecophenotypes) and demand additional taxonomic examination using modern techniques. These presumed species are often difficult to distinguish based on shell alone, and the shell was the source of the prime characters used by Henderson & Bartsch. Henderson & Bartsch also identified a very large number of small mollusks they defined as new; many of these are now regarded as synonymies of other species. It is clear that the cluster of species described by Henderson & Bartsch is in dire need of re-examination in the future.

Taxonomic uncertainties, however, are not limited only to the small mollusks. Neither the present study nor other recent studies isolate *Spisula solidissima similis* as a valid subspecies of *S. solidissima*. This species is under taxonomic evaluation. *S. raveneli*, an example of the taxonomic problems within this genus, is found in the southernmost range of *S. solidissima* but of questionable species validity (Cargnelli et al., 1999) as well. We are probably looking at a series of ecophenotypes along the North American eastern seaboard. It is clear that there are taxonomic issues in all the lists used in this study and that many critical issues, at least at the species level, remain to be resolved.

The variability and large array of microhabitats, small size of many of the mollusks, temporal variability of some habitats (fringing marshes, seagrass beds, etc.),

cryptic habits of some species, continued changes to and additions of artificial substrata (i.e., new piers, docks, pilings, etc.), examination of previously unexplored subtidal sites, and relative collection efforts among the various studies, could account for at least some of the differences among the studies. These same factors ensure that additional species will be found in the future. Certainly the creation and loss of inlets cutting through the island influences circulation, salinity, temperature, and predator access, and could thus shift species distributions. Major shifts in salinity altered the region's previously extensive oyster beds and coincidentally its associated fauna. For a review see Counts & Bashore (1991) and Homer et al. (1997).

#### Study Similarities and Differences

There are notable differences and similarities between and among the primary studies examined in this paper. Table 4 shows the uniqueness of specific studies as defined as species collected in one study and not a comparable study. Ignoring taxa listed as possibly new by Henderson & Bartsch (1914) and unidentified by Homer et al. (1997), the largest difference is between the present study and that of Henderson & Bartsch (1914). For instance, we collected 31 species of gastropods not listed

Table 5

Overlap of species between collections (Henderson & Bartsch [1914], Counts & Bashore [1991], Homer et al. [1997] and Present Study). The last column represents the total number of molluscan species (including cephalopods and chitons) found in common between the two studies noted in the left column.

	Bivalves	Gastropods	Total
Henderson & Bartsch and Counts & Bashore	22	26	50
Henderson & Bartsch and Homer et al.	20	24	44
Henderson & Bartsch and Present Study	27	27	57
Counts & Bashore and Homer et al.	23	29	54
Counts & Bashore and Present Study	26	32	60
Homer et al. and Present Study	28	29	60



Table 6

Similarity indices using Bray-Curtis analysis for molluscan taxa recovered from the present study and those of Henderson & Bartsch (1914), Counts & Bashore (1991) and Homer et al. (1997). A. Indices for all molluscan fauna. B. Indices for bivalves only. C. Indices for gastropods only.

	Henderson & Bartsch (1914)	Counts & Bashore (1991)	Homer et al. (1997)
<b>A. ALL MOLLUSKS</b>			
Counts & Bashore	62.338	xxxxx	xxxxx
Homer et al.	60.274	62.319	xxxxx
Present study	57.447	66.667	66.279
<b>B. BIVALVIA</b>			
Counts & Bashore	67.768	xxxxx	xxxxx
Homer et al.	58.824	73.016	xxxxx
Present study	64.286	65.823	71.795
<b>C. GASTROPODA</b>			
Counts & Bashore	63.415	xxxxx	xxxxx
Homer et al.	61.539	55.556	xxxxx
Present study	54.000	65.957	64.444

by Henderson & Bartsch. Perhaps of equal importance in total analysis is the similarity of species found between studies (Table 5). The highest similarity of bivalves (same species collected) can be found between Homer et al. (1997) and the present study. Counts & Bashore (1991) and the present study matched with a total of 60 species of mollusks.

The Bray-Curtis Index of Similarity was used to compare presence/absence and overlap of molluscan species between and among the four studies. Similarities between each pair of collections can also be found in Table 6. A comparison of degree of similarity between the present and each previous study can be found in the hierarchical clusters in Figure 2 (including total malacofauna and bivalves and gastropods examined separately). As can be seen in the clusters in Figure 2A, for overall mollusks the present study most closely aligns with Counts & Bashore (1991), and the latter two form a cluster with Homer et al. (1997). Only Henderson & Bartsch (1914) remain isolated with a total similarity index to all the other studies of about 65. For bivalves alone, Homer et al. (1997) cluster with Counts & Bashore (1991), and these two then form a cluster with the present study. Again, Henderson & Bartsch (1914) remain isolated. However, for gastropods alone, the present study most closely aligns with Counts & Bashore (1991).

The greatest similarity for all mollusks collected resides among the present study, Counts & Bashore (1991) and Homer et al. (1997) [similarity = 66.667 and 66.279, respectively]. Henderson & Bartsch (1914) and the present study had a similarity index of 57.447. Thus the earliest and the latest studies had the lowest degree of sim-

ilarity, whereas the three more recent studies, with collections entailing more extensive effort and duration, were more similar. In the earliest study, the fauna were collected over a shorter period of time and using only the shore as the staging area for collections. The greatest similarity in bivalves collected was found between Counts & Bashore (1991) and Homer (1997) (similarity index = 73.016) (Table 6B). However, Counts & Bashore (1991) and the present study showed the highest similarity in gastropods collected (similarity index = 65.957) (Table 6C). The latter could reflect the careful perusal of varied habitats by these authors but more likely reflects the relatively lower number of gastropods by Counts & Bashore (1991) (39) with most being the more common species encountered. Additionally, the Bray-Curtis index does not overly weigh the overlap of 32 species (Table 5) and the relatively small number (seven; Table 4) of unique species collected by Counts & Bashore compared to the present study. Recall that the present study collected 108 species in toto compared to the next highest number of species collected (81 by Henderson & Bartsch, 1914). Thus the large number of non-overlapping taxa could create the lower levels of similarity in the newer studies when compared to each other or the earlier surveys. A large number of overlapping species could create the higher levels of similarity in the newer studies outweighing additional non-overlapping species.

#### Range Extensions and New Records

Discovery of hitherto unrecorded taxa in this region is not surprising. The very limited number of surveys along this coast easily accounts for some of the variances in molluscan taxonomic lists. The range extensions noted herein (Table 7) can also be accounted for by the dearth of published faunal surveys of this region. Some species found in the present study but not recorded by Counts & Bashore (1991) or Henderson & Bartsch (1914) are readily related to collection effort; the present study ran a course of several years and had an extended and large (though variable) collection team. Henderson & Bartsch made their collection during 1 week. Additionally, present collections included benthic surveys from slightly deeper waters not accessible to the former authors. In some cases, the small size and relative rarity of the species demanded intensive collection efforts or luck. For instance, the small lasaeid bivalve *Aligena elevata* was found in very shallow (wading) water of Tom's Cove, Assateague Island, Virginia only once. *Aligena elevata* is sometimes associated with the parchment tube of the polychaete *Diopatra cuprea*, a species quite common along the edge of Tom's Cove. It is possible that intensive sampling of the home tubes of these polychaetes would turn up additional specimens.

Homer et al. (1997) noted northern extensions in the ranges of three mollusks. *Pyramidella crenulata*, first

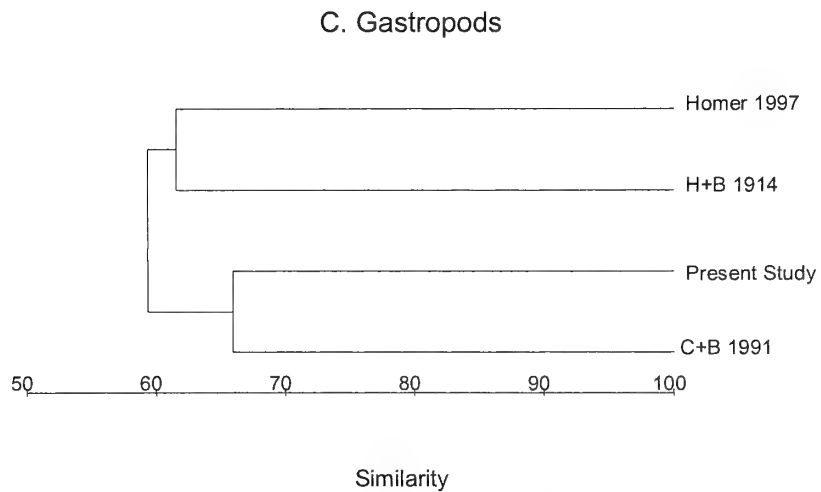
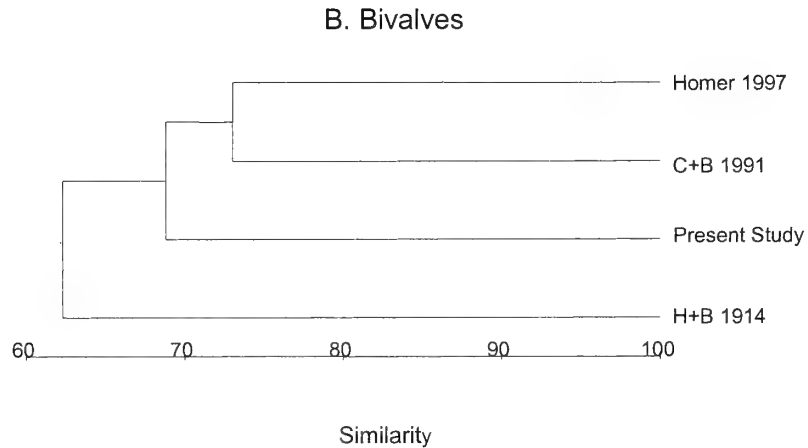
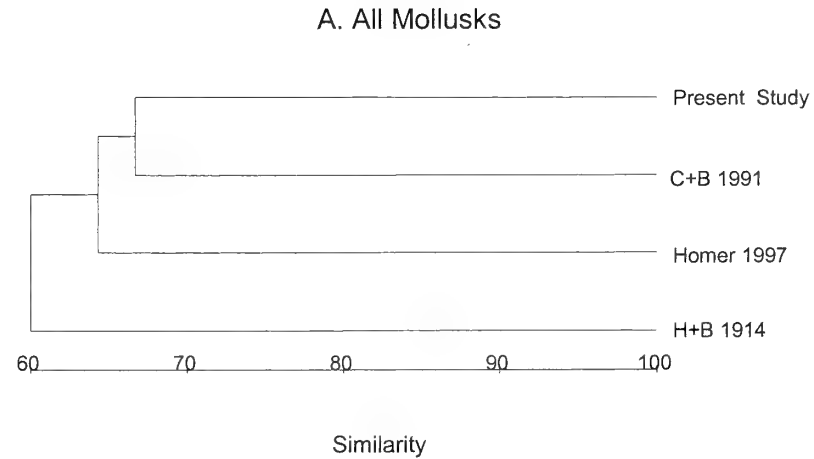


Figure 2. Hierarchical cluster analysis of the present study and Henderson & Bartsch (1914) ("H+B 1914"), Counts & Bashore (1991) ("C+B 1991"), and Homer et al. (1997) ("Homer 1997") using Bray-Curtis similarity indices. Figure 2A clusters studies using all molluscan taxa recovered. Figure 2B clusters for bivalves only; Figure 2C clusters for gastropods only.

Table 7

Species (mollusk and non-mollusk) from Assateague and Chincoteague Islands with range extensions. Source of previous range is noted. Two web sites are given: Fautin 2001: [http://deuteron.kgs.ukans.edu/KWRC/anemone2/classification/current\\_classification.htm](http://deuteron.kgs.ukans.edu/KWRC/anemone2/classification/current_classification.htm) and Hardy 2001: [http://www.gastropods.com/shell\\_pages/index.html](http://www.gastropods.com/shell_pages/index.html)

Species	Previously reported range
<b>BIVALVIA</b>	
<i>Pleuromeris tridentata</i>	North Carolina to all of Florida (Abbott, 1974); unconfirmed collection from "Long Island," New York in field guide of Long Island shells
<i>Tridonta borealis</i>	Arctic seas to Massachusetts Bay (Abbot, 1974)
<b>GASTROPODA</b>	
<i>Eupleura sulcidentata</i>	Florida, Bimini (Abbott, 1974; Lyons, 1977; Hardy, URL above)
<i>Kurtziella limonitella</i>	North Carolina to both sides of Florida; Jamaica (Abbott, 1974; Hardy, URL above)
<i>Tectura testudinalis</i>	Arctic seas to Long Island Sound, New York (Abbott, 1974)
<b>ANTHOZOA</b>	
<i>Peachia parasitica</i>	Adult anemones of this species typically not found south of Eastport, MA (Gosner, 1971); oceanic along northernmost coast of eastern United States up into southern Canadian coast (Fautin, URL above); occurrence noted in lower Chesapeake Bay (McDermott et al., 1982); associated with sub-umbrella of scyphozoan medusae
<b>ASCIDIACEA</b>	
<i>Ecteinascidia turbinata</i>	South Florida to Texas (Meinkoth, 1981; Plough, 1978); live-bearing tunicates

found in Chincoteague Bay in 1981 by Casey & Wesche (1982), was previously known only north to North Carolina (Abbott, 1974). *Cyclinella tenuis* was found in Chincoteague Bay (Homer et al., 1997) and considered a range extension from Cape Hatteras. Boss & Wass (1970), however, found this species in the lower Chesapeake Bay. Lastly, Homer et al. (1997) found *Acteocina bidentata* in Chincoteague Bay, whereas it was previously recorded north only to North Carolina (Abbott, 1974). The latter is somewhat confusing as the taxonomy has changed and it is assumed that Henderson & Bartsch (1914) had found this snail in their work but listed it under an earlier name.

Some of the noted range extensions in our study, as in that of Homer et al. (1997) are minor and not surprising. It is also true that publications denoting range extensions are today relatively infrequent. Conceptually historical zoogeographic boundaries, while still a useful concept in distribution studies, are often "ignored" by otherwise isolated taxa; this is especially true for species with a narrow distribution and nearby congeners. Some extensions, however, have evident significance. We found the limpet *Tectura testudinalis* in Cockle Creek, Chincoteague, Virginia on oyster rubble at about 4 m depth. Previously the southernmost known range of this limpet was Long Island Sound, New York. All specimens of this limpet found in Virginia waters were quite small and subtidal. In this case, the small size could reflect relatively young specimens that had yet to survive an entire year in this southern location. Additionally, the subtidal location is not unusual for a southern range extension since this would represent cooler and perhaps more tolerable waters for the typically northern species.

*Eupleura sulcidentata* was found in Mosquito Creek,

Chincoteague, at a depth of about 2 m on rocky rubble and also intertidally on shell and rock rubble in Memorial Park, Chincoteague. This represents a significant range extension; *E. sulcidentata* is typically found along the west coast of Florida and Bimini (Lyons, 1977). Lyons (1977) suggested that Lake Worth Inlet on the Florida east coast could be the northernmost range limit for this species. Various species associated with oyster beds are frequently transplanted along with oysters, and this is a possible route for this gastropod to the Maryland and Virginia coast. More typical extensions can result from occasional shifts in prevailing coastal currents. This is demonstrated by the rare appearance of the scyphozoan dwelling anemone *Peachia parasitica* (Agassiz, 1859) from the north and the viviparous ascidian *Ecteinascidia turbinata* from the south in the central location of Chincoteague. Although as recently as 2001, D. G. Fautin noted in an on-line database ([http://deuteron.kgs.ukans.edu/KWRC/anemone2/classification/current\\_classification.htm](http://deuteron.kgs.ukans.edu/KWRC/anemone2/classification/current_classification.htm)) this "oceanic" anthozoan as being distributed in the extreme northeast of the United States and up along the Canadian coast, *Peachia parasitica* had been recorded from the lower Chesapeake Bay in the early 1980s (McDermott et al., 1982) as a "symbiont" of *Cyanea capillata*.

#### "Gigantism"

Rex & Etter (1997) discussed a recent spate of papers showing a renewed interest in the question of body size. Body size has clear implications in ecological and evolutionary studies, with these perhaps serving as the stimuli for the resurgence in size studies. Variation in intra-specific size is most commonly attributed to a combination of parameters that include diet, competition, preda-

Table 8

Molluscan species demonstrating “gigantism” (or exceptionally large size) at Assateague Island. Dimensions of Assateague specimens from present study or as noted. Size of specimens denoted from literature have not been verified here. True “giants” (here defined as at least 25% larger than previously recorded maximum size) are indicated with an asterisk.

Species	Previously reported dimensions	Dimensions of assateague specimens
<b>BIVALVIA</b>		
* <i>Lyonsia hyalina</i>	12.68 to 19.02 mm shell length (Abbott, 1974)	25.5 mm shell length, 14.2 mm shell height; rate in area
<b>GASTROPODA</b>		
<i>Bittium alternatum virginicum</i>	Possibly a giant ecological form of <i>Bittium alternatum</i> (Say, 1822); shell height of 6.3 mm (Abbott, 1974)	Exceptionally large specimens of 8.3 mm from Chincoteague recorded in Henderson & Bartsch (1914) as <i>Diastoma virginica</i> n. sp.
<i>Busycon carica</i>	125.85 to 228.33 mm in length (Abbott, 1974) (but recorded at 281 mm from S. Carolina in Hutsell et al. 1999)	253 mm shell length
* <i>Crepidula convexa</i>	6.34 mm to 12.68 mm (Abbott, 1974)	24.5 mm
<i>Crepidula fornicata</i>	19.02 to 51.74 mm (Abbott, 1974) (but recorded at 66.7 mm (no locality data) in Hustell et al., 1999)	Many large specimens, largest being 58.7 mm; all female stage
<i>Urosalpinx cinerea folleyensis</i>	68.3 mm from Washington noted (Hutsell et al., 1999)	Exceptionally large <i>Delmarva</i> specimens reported by Baker (1951) and from Chincoteague by Henderson & Bartsch (1914)
<b>POLYPLACOPHORA</b>		
* <i>Chaetopleura apiculata</i>	7 to 20 mm in length (Abbott, 1974)	30.5 and 40.0 mm in length, four remarkably large specimens; rare in area

tion, environmental energetics (i.e., high wave intertidal versus quiescent mud flat), population density, offspring size, temperature, and other environmental variables (Branch & Branch, 1980; Branch, 1981; Underwood, 1984a, b; Bowling, 1994; Schindler et al., 1994; Sibly & Atkinson, 1994; Strayer, 1994; Atkinson, 1995; Takada, 1995; Kozlowski, 1996; Yampolosky & Schneiner, 1996). Homer et al. (1997) noted that coastal bay populations of *Nassarius vibex* tend to reach larger sizes than usually reported (they found that *N. vibex* in Chincoteague Bay averaged 15.8 mm long with a range between 9.0–18.0 mm, whereas “shell guides” typically report them to be smaller). Causative effects of within-habitat variation in size of relatively sedentary mollusks can reflect tide level and differences in microhabitat (Sutherland, 1970; Creese, 1980; Fletcher, 1984; Takada, 1995). Öst & Kilpi (1997) and Kautsky (1982) discussed a variety of environmental parameters that influence the maximum size of the blue mussel *Mytilus edulis* in Baltic waters. These include temperature, salinity, wave and light exposure, food supply, and population structure. Parasitism is also an occasional cause of gigantism in some snails (Mouritsen & Jensen, 1994). For example, trematodes of “low-pathogenicity” have been shown to cause gigantism in *Hydrobia* spp. (Gorbushin, 1997). De Jong-Brink (1995) suggested that trematodes could influence neuroendocrine functions in snails (in this case *Lymnaea stagnalis*) and induce increased growth. Baker (1951) reported a case of “gigantism” of *Urosalpinx cinerea folleyensis* from

Chincoteague. Similarly, Henderson & Bartsch (1914) noted the “enormous size” of specimens of this gastropod taken from Chincoteague oyster beds. In the present study, we found exceptionally large specimens of two species of bivalve, four species of gastropod, and the chiton *Chaetopleura apiculata* (Table 8). These mollusks range from filter feeders (*Lyonsia hyalina* and *Crepidula convexa*) to grazing herbivores (*Chaetopleura apiculata*) to predatory carnivores (*Busycon carica*). None were found in particularly large numbers and there was little evidence of disproportionately intense predation of smaller cohorts of the filter feeders or grazers, although only large *Chaetopleura apiculata* and *Lyonsia hyalina* were found. The list includes both infaunal and epifaunal species, subtidal and intertidal species, and common and rare species. Nevertheless, *L. hyalina* and *C. apiculata* are noteworthy for their extreme sizes.

Tablado et al. (1994) found that the pulmonate limpet *Siphonaria lessona* grew to larger sizes in sewage polluted areas of Argentina. These authors suspected the cause of larger snails was either directly or indirectly a result of organic enrichment. The anthropogenic input in the Assateague region is relatively high, especially during summer tourist season. However, the larger specimens found in our study represented only a small minority of the total population (except for *Chaetopleura apiculata*, which had an overall small population), and it seems unlikely that any general environmental factor could be causative.

Recent studies by Rex & Etter (1997) showed that both

larval and adult shells increase in size with depth into the abyss. They suggested that the decreased input of nutrients into these deep waters will select for the larger specimens that would have a competitive or metabolic advantage. Whether this is accurate or not, it is clear that location plays a significant role in size distribution. Chincoteague and Assateague are in the Boreal province and Virginian subprovince. The region offers a wide array of temperate marine and estuarine habitats that are not as warm as those to the south or as seasonally cool as those to the north. While not a delineated provincial zone, this region offers a blend or transition between the Carolinian province and the Boreal. Although Cape Hatteras is the identified division between these provinces, temporal variations in the Gulf Stream can bring decisively Carolinian fauna up along Assateague. Similarly, shifts in the Labrador Current can bring cooler water species south. Such displaced species are common along northern coastal Virginia and southern Maryland (see list of range extensions; also note that during these collections several species of semitropical fish [e.g., *Chaetodon*] were found along our field sites). Does the integrative nature of this region influence growth rates or longevity as well as allowing an out-of-range existence?

The possibility, in all studies that reveal "gigantism," that sampling artifact plays a role cannot be overlooked. It is easy to envision an artificial selection of larger specimens in any collection. Here, however, our collections were over many years, and the "giants" included species that are considered uncommon in the region (e.g., *Lyonsia hyalina*, *Chaetopleura apiculata*). The extensive sampling (in terms of number of individuals doing the surveys plus time allotments) would certainly have revealed larger populations of these species through time. In most cases, the specimens of a particular species, large or not, were only rarely collected. Smaller specimens of the same species were equally atypical in these communities. Along the opposite spectrum, Prezant (1979, 1981) reported a "dwarfed" population of *Lyonsia hyalina* from Nahant Bay, Massachusetts. This population was composed of significantly smaller individuals, averaging half or less the size of those from more southerly populations (e.g., Cape Cod). The exact reason for this smaller size was not determined; however, the Nahant population was consistently infected with dense populations of coccidia that almost occluded the proximal limbs of their kidneys. In this case, as opposed to the "gigantism" apparently induced by trematode-infected *Hydrobia* (Gorbushin, 1997), it is possible that a parasitic infestation reduced maximum growth attained.

High seasonal primary productivity, coupled with the large array of protected natural and manmade habitats, offers conditions for a rich and stable food supply. The question then is, at least in part, not why a few species in this region have a few specimens that are large, but why the hundreds of other species lack these unusually

large representatives and why so few within a population grow to unusually large sizes? Aside from the obvious ease with which the larger specimens are found, the answer probably rests with a few genetic anomalies confined within overall genetic constraints.

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## NOTES, INFORMATION & NEWS

### The Century's Finest

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At the beginning of the last century the malacological world was privileged to have an array of distinguished practitioners. William H. Dall and Paul Bartsch at the Smithsonian Institution, Henry A. Pilsbry at the Academy of Natural Sciences in Philadelphia, Harold Heath at Stanford University, S. Stillman Berry in Redlands, California, and numerous others led the way in describing the living and fossil molluscan fauna of North America. These workers ultimately described over 10,000 taxa, and their efforts capped what might be thought of as the “Golden Age” of American malacology. However, the most influential malacologist of the twentieth century would not be born for another 40 years. In contrast, he would describe only a handful of taxa in a career that spanned the last half of the century, but there is no denying the import of his contributions to the field of malacology and far beyond.

Stephen Jay Gould was born on September 10, 1941, in Queens, New York. Like many students of natural history his fascination with organisms began at an early age, and the dinosaur exhibit in the American Museum of Natural History in New York was a favorite destination. Steve obtained his undergraduate degree in Geology at Antioch College, and went on to graduate work at Columbia University, receiving his Ph.D. in 1967. However, the question he chose for his dissertation was not in deep time but rather in the shallow sand dunes of Bermuda. Steve had become fascinated by the diversity of land snails there and in the Bahamas and he sought to understand their insular evolutionary patterns. Papers on *Poecilozonites* and *Cerion* soon followed, many co-authored with David Woodruff. In 1984, Steve described his first two species—the Giant and Dwarf Smokestack *Cerion* (*Cerion excelsior* Gould, 1984a, and *Cerion caminus* Gould, 1984a, respectively).

From his study of Bahaman land snails Steve noticed that morphological evolution in *Poecilozonites* was not gradual; rather, large changes appeared suddenly, and these morphological reorganizations were short lived in the fossil record and followed by another period of stasis. Another Columbia University graduate student had noticed a similar pattern in the diversification of trilobites, and after comparing notes they joined forces as Eldredge & Gould (1972) to unleash punctuated equilibrium on a paleontological world unaware of its reliance on a cloven hoof print of theory—gradualism. To be certain, the pres-

ence of stasis in the fossil record had been noticed much earlier (e.g., Dall, 1877), but rather than eschew it as artifact (or use it to argue against Darwinian evolution), Eldredge and Gould embraced it as the fossil signature of allopatric speciation and extended its implications into macroevolution theory.

In 1977 *Ontogeny and Phylogeny* was published. This seminal volume recovered the baby that had been thrown out with Haeckel's bathwater, and foreshadowed the resurgence of the field of evolutionary development. It also had a profound influence on a cohort of graduate students who read the book in seminars across the country. Molluscan examples were scattered throughout the text, including Ockelman's (1964) study of small insular bivalves, Stanley's (1972) progenetic transitions in bivalve habits, Hoagland's (1975) dissertation work on life history evolution in *Crepidula*, as well as Steve's own work on *Poecilozonites* and *Cerion*.

It is not surprising that mollusks also figured prominently as study organisms among Steve's students. These students included Warren Allmon (1988) who investigated heterochrony in the evolution of *Turritella* shell morphology, Dana Geary (1986) who studied a Late Miocene radiation of melanopsid gastropods, and Jane Rose (1990) who examined the relationship between ecology and variation in *Cerion*. Many of his students' themes were familiar, the relationships between ontogeny and phylogeny, and comparisons of punctuated vs. gradual patterns of diversification. Where necessary, there was a sophisticated array of statistical and multivariate analyses to quantify morphology and search for patterns through time. Steve often had an impressive multivariate methodology in his own work (e.g., Gould, 1967, 1970, 1984b) and his rigorous quantitative approach was mirrored in the work of many of his students.

It is also well known that Steve was not a “computer geek,” and many obituaries have commented on his avoidance of word processors and POP3 compliant programs. I also doubt that Steve ever navigated PAUP\* or MacClade, but his own personal aversions never limited his students' research programs; for example phylogenetic analyses were prominent in the work of Morris (1991) and Yacobucci (1999).

Mollusks also served as exemplars in Steve's column “This View of Life” that appeared in the pages of *Natural History Magazine*. His commentaries dealt with natural history issues that ranged from hens' teeth to the dating of the beginning of the millennium; and mollusks often graced those pages as well. In fact, the story of an extinct little limpet once even found its way into a column! However, the importance of those articles (and their



afterlives in collected volumes) should not be underestimated for they translated the esoteric reports of our research into popular pieces that have so far entertained and educated two generations of lay naturalists.

Although Steve's presence in the twenty-first century will be remembered as fleeting, this century will be marked by his greatest contribution, his *magnum opus*—*The Structure of Evolutionary Theory* (2002). Steve's view of evolution as outlined in 1433 pages is (as it ever was) pluralistic and hieratical, and for that he took substantial criticism from fundamental Darwinians and others (Morris, 2001). Steve's ideas (as well as his prose) often exasperated some while inspiring others and this book is no different. David Wake (2002) has predicted that *The Structure of Evolutionary Theory* "... will be a permanent factor in the struggle to understand how life has evolved." Like Steve's other writings, *The Structure of Evolutionary Theory* contains numerous molluscan examples supporting his view of the history of life—from the punctuated evolutionary patterns of melanopsid speciation to the spandrels of trochacean brooding.

Steve Gould's death on May 20, 2002, ended the career of the last century's finest malacologist, but his legacy to malacology is immense. Steve never produced a classic monograph or performed cladistic analyses that spawned cascades of nomenclatural changes, and his name will appear as taxon author on only a few leaves on the tree of life. Nevertheless, his contribution to our field transcends all of these conventional measures. Steve showed us how mollusks could be used to unravel the patterns and processes of the last half a billion years of life, and when current theories and models failed to explain these patterns, Steve was not shy about proposing new ones.

In fairness, Steve was not the only one to travel down this path. His cohort includes such eminent colleagues as Michael Ghiselin, Steve Stanley, and Geerat Vermeij—malacologists all—who have extended our collective vision beyond the usual taxon-based questions and practices that we typically undertake. They took our (and their) favorite taxon and addressed a broad suite of evolutionary questions that provide insights into some of the processes that have shaped the history of life on earth, and they shuffle shells (and the shell-less) with the best of them. I observed Steve during a visit he made to Berkeley in 1988 move effortlessly through our *Cerion* holdings, suggesting mixed lots and re-identifying specimens. He also searched the vermetids for especially meandering specimens. Their openness intrigued him, rules were being broken, and the transition seemed to mark an important yet unknown event in both their ontogeny and phylogeny. He clearly understood and relished the value of museum collections and was just as accomplished there as he was penning an introduction to a research paper that would cast *Achatinella* as the devil's advocate and the Rev. Gulick as Mephisto (Gould, 1971).

One cannot help but notice the parallels between the

turn of the last century and today. The malacological contributions and scope of work by Dall, Pilsbry, Berry, and others in the early 1900s were enormous and often viewed as insurmountable by later workers. Up until about 20 years ago, most American malacological polls would have undoubtedly chosen one of these gentlemen's contributions as the most significant of the twentieth century. Today the work of Gould and others has shown us the potential of molluscan studies, and set new standards and expectations for modern malacological research. However, I doubt that Steve will vie for first place at the end of the current century. That spot will likely be reserved for a malacologist who has yet to undergo meiosis. We cannot predict where his or her future contributions may lie. I suspect that assembling the unfalsified Mollusca branch of the tree of life or determining the regulatory cascades of the key innovations in the diversification of the molluscan *bauplan* will certainly occur in the next 98 years. However, since we cannot know our future intellectual descent's contingencies we have no way to predict the directions of that future research. Therefore we might as well just get on with the work before us—Steve would have it no other way.

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**Anatomical Description of *Psidium johnsoni*  
E.A. Smith, 1882 (Bivalvia: Sphaeriidae)  
from Madagascar**

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Kuiper (1966) reported five species of the genus *Psidium* C. Pfeiffer, 1821, from Madagascar, one of them [*P. casertanum* (Poli, 1791)] being cosmopolitan, two (*P. ovampicum* Ancey, 1890, and *P. viridarium* Kuiper, 1956) representing the African fauna, and two (*P. johnsoni* E.A. Smith, 1882, and *P. betafoense* Kuiper, 1953) being restricted to the island. *P. johnsoni* was the most interesting among these species, since its similarity to the Holarctic *P. milium* (Held, 1836) was noted (Kuiper, 1966). However, no anatomical data on the species were available until now, whereas soft body characters have proved to be rather informative for the systematic and phylogenetic studies in Palearctic and African Sphaeriidae (Korniushev, 1998a, b).

Recently, we examined a sample from Central Madagascar, now deposited in the Field Museum of Natural History, Chicago (FMNH), containing two *Psidium* species. One of the species (FMNH 296603) was identified as *P. viridarium*, and its anatomical characters were in good agreement with those reported in the literature (Korniushev, 1998b). The other species (now FMNH 296604)

appeared to be *P. johnsoni*, and a description of its anatomy is provided below. The species identification was confirmed by comparison with the lectotype of *P. johnsoni* deposited at the Natural History Museum, London (BMNH) and examined by the senior author in 1995.

For comparison, materials from the collections of D. S. Brown (*Psidium ovampicum*) and A.V. Korniushev (*P. milium*) were used.

All samples were preserved in alcohol. Anatomical characters were observed under a stereomicroscope and drawn with a camera lucida. Gill and mantle preparations were processed according to Korniushev (1995).

Below a description of the anatomical characters and a brief discussion of the possible relationships of the examined species are provided.

*Psidium johnsoni* E.A. Smith, 1882

**Material:** Lectotype BMNH 82.3.5.23, 20 lieu (about 80 km) from Tananarivu, Madagascar; FMNH 296604, 1 km N of Ilempona, approx. 40 km NE of Antsirabe, Central Madagascar, in a shallow ditch along railroad tracks, leg. R. Webrantz 15 December 1989, 3 specimens.

**Shell characters (Figures 1A, B):** Specimens FMNH 296604 corresponding with the published description (Kuiper, 1966) and the lectotype.

**Adductor muscles:** Posterior adductor small, oval (Figure 1C). Anterior adductor bean-shaped, markedly shifted upward (dorsally).

**Mantle:** Mantle edge thickened by strong development of longitudinal muscles (Figures 1C, F). Presiphonal suture markedly elongated, longer than pedal slit. Inner radial mantle muscles arranged in four strong and clearly defined bundles, three of them (anterior) placed at edge of pedal slit close to each other, posterior bundle at distal end of presiphonal suture.

**Gill:** Outer demibranch placed at tenth filament of inner one (two specimens examined). Brood pouch in low position (Figure 1D), formed by four filaments of inner demibranch and partly covered by the inner (ascending) lamella. Three large larvae found in each of studied pouches.

**Nephridium:** Open type (pericardial duct visible between branches of dorsal lobe), dorsal lobe quadrangular (Figure 1E).

**Remarks:** The elongated presiphonal suture and very short pedal slit were noticeable also in the dried soft body of the lectotype (Figure 1G).

*Psidium johnsoni* has a very peculiar anatomy and is distinctly different from other species of *Psidium*. However, it is similar to the Holarctic *P. milium* (see Korniushev, 1996) in its very short pedal slit, in addition to the shell characters reported by Kuiper (1966). A similar anatomy was also reported for the Madagascar and Af-

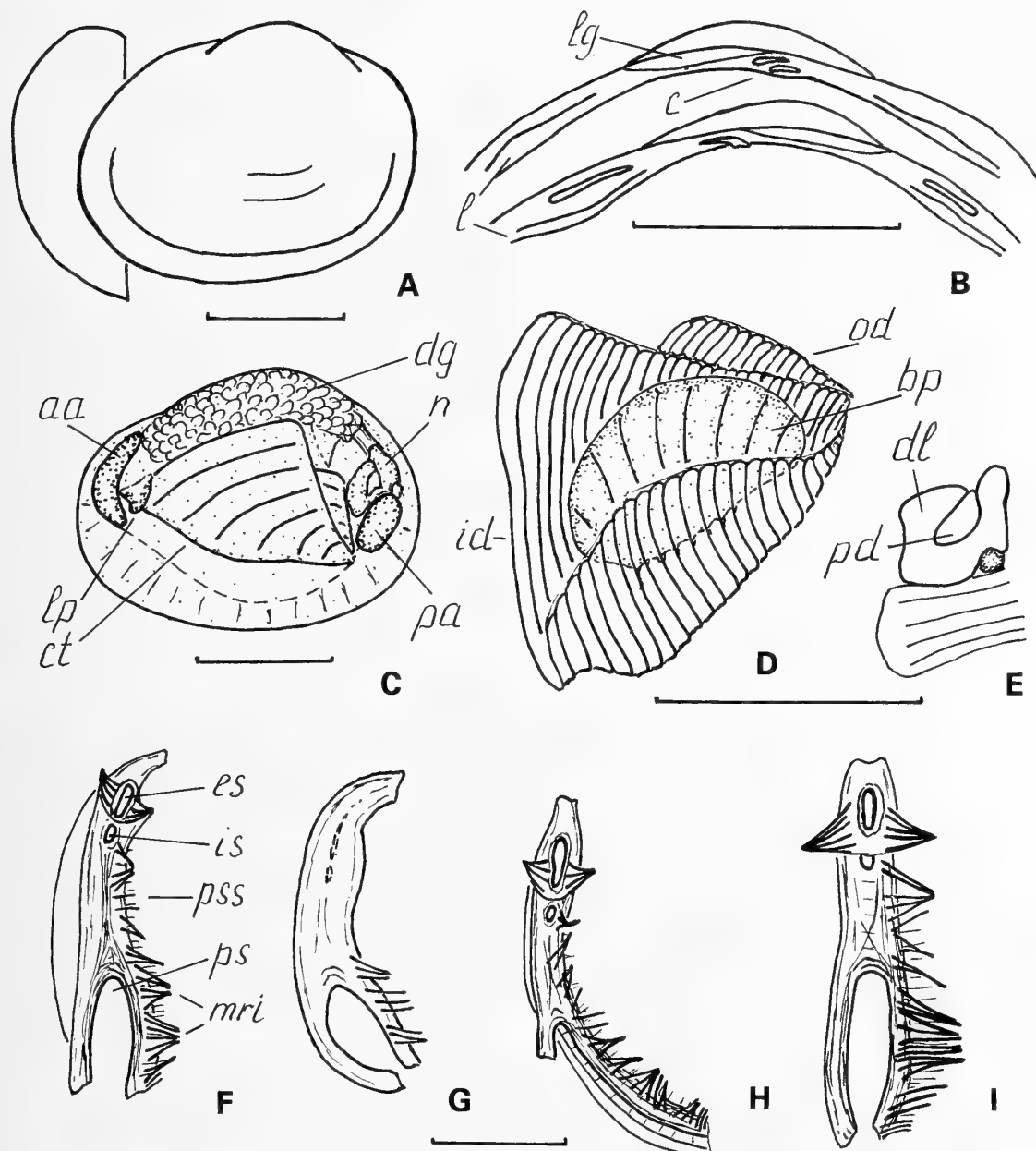


Figure 1. A–F Shell and anatomy of *Pisidium johnsoni* from Madagascar (FMNH 296604). A. Lateral and frontal view of shell. B. Hinge (above—left valve, below—right valve). C. Gross anatomy. D. Gill from inner side. E. Dorsal view of nephridium. F. Mantle edge from inner side. G. Lectotype of *P. johnsoni* (BMNH 82.3.5.23), mantle edge of dried soft body from outside. H. *Pisidium ovampicum*, South Africa, D. Brown collection, mantle edge from inner side. I. The same structure in *Pisidium milium*, Ukraine, A. Korniushev collection. Key: aa, anterior adductor; bp, brood pouch; c, cardinal teeth; ct, ctenidium; dg, digestive gland; dl, dorsal lobe; es, exhalant siphon; id, inner demibranch; is, inhalant siphon (opening); l, lateral teeth; lg, ligament; lp, labial palps; mri, inner radial mantle muscles; n, nephridium; od, outer demibranch; pa, posterior adductor; pd, pericardial duct; ps, pedal slit; pss, presiphonal suture. Scale bars = 1 mm.

rican species *P. ovampicum* (see Korniushev, 1998b). The three species under consideration share the following anatomical characters: bean-shaped anterior adductor, markedly elongated presiphonal suture, concentrated inner radial mantle muscles, and open type of nephridium. *Pisi-*

*dium johnsoni* is unique in the strong development of its longitudinal mantle muscles, especially in the presiphonal suture. *Pisidium milium* differs from *P. johnsoni* in the lower placement of its anterior adductor, as well as in much more pronounced and more concentrated inner ra-

dial mantle muscles (Figure 1I). Concerning the arrangement of muscle bundles, *P. johnsoni* is more similar to *P. ovampicum* than to *P. milium* (Figure 1H).

Korniushin (1998a) suggested that an elongated presiphonal suture and concentrated radial mantle muscles were apomorphic character states among species of *Pisidium*. Also, the large bean-shaped adductor has not been observed in the other studied species of this genus. Thus, at least some of the similarities between the three discussed species may be synapomorphies supporting their belonging to a separate clade within *Pisidium*. However, their relationships cannot be resolved now. Either *P. milium* and *P. johnsoni*, or, under the assumption that the strong similarities of the latter two species are resulting from parallel evolution, *P. ovampicum* and *P. johnsoni* might be regarded as a pair of sister taxa. A phylogenetic analysis of the whole genus *Pisidium* is needed in order to support or reject these hypotheses.

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Manuscripts must be typed, one side only, on A4 or equivalent (e.g., 8½" × 11") white paper, and double-spaced throughout, including references, figure legends, footnotes, and tables. All margins should be at least 25 mm wide. Number the pages. Text should be ragged right (i.e., not full justified). Avoid hyphenating words at the right margin. Manuscripts, including figures, should be submitted in triplicate. The first mention in the text of the scientific name of a species should be accompanied by the taxonomic authority, including the year. Underline scientific names and other words to be printed in italics; no other manipulation of type faces is necessary on the manuscript. Metric and Celsius units are to be used. For aspects of style not addressed here, please see a recent issue of the journal.

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In most cases, the parts of a manuscript should be as follows: title page, abstract, introduction, materials and methods, results, discussion, acknowledgments, literature cited, figure legends, footnotes, tables, and figures. The title page should include the title, authors' names, and addresses. The abstract should be less than 200 words long and should describe concisely the scope, main results, and conclusions of the paper; it should not include references. An abstract should "show, not tell"; e.g., not "lengths were recorded" but "lengths ranged from 5.0 mm to 7.5 mm."

## Literature cited

References in the text should be given by the name of the author(s) followed by the date of publication: for one author (Phillips, 1981), for two authors (Phillips & Smith, 1982), and for more than two (Phillips et al., 1983).

The Literature Cited section should include all (and only) references cited in the text, listed in alphabetical order by author. No citation is needed when author and date are given only as authority for a taxonomic name. Each citation must be complete, with all journal titles *unabbreviated*, and in the following forms:

### a) Periodicals:

Hickman, C. S. 1992. Reproduction and development of trochacean gastropods. *The Veliger* 35:245–272.

### b) Books:

Bequaert, J. C. & W. B. Miller. 1973. *The Mollusks of the Arid Southwest*. University of Arizona Press: Tucson. xvi + 271 pp.

### c) Composite works:

Feder, H. M. 1980. Asteroidea: the sea stars. Pp. 117–135 in R. H. Morris, D. P. Abbott & E. C. Haderlie (eds.), *Intertidal Invertebrates of California*. Stanford University Press: Stanford, Calif.

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Figures must be carefully prepared and submitted ready for publication. Each should have a short legend, listed on a sheet following the literature cited. Text figures should be in black ink and completely lettered. Keep in mind page format and column size when designing figures. Photographs for halftone reproduction must be of good quality, trimmed squarely, grouped as appropriate, and mounted on suitably heavy board. Where appropriate, a scale bar may be used in the photograph; otherwise, the specimen size should be given in the figure legend. Photographs should be submitted in the desired final size.

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Use one consecutive set of Arabic numbers for all illustrations (that is, Figures 1, 2, 3..., *not* Figure 1a, 1b, 1c..., *nor* Plate 1, fig.1...).

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Each manuscript is critically evaluated by at least two reviewers. From these evaluations the editor makes a preliminary decision of acceptance or rejection. The editor's decision and the reviewers' comments are sent to the corresponding author for consideration and further action. Unless requested, only one copy of the final, revised manuscript needs to be returned to the editor. The author is informed of the final decision and acceptable manuscripts are forwarded to the printer. The author will receive proofs from the printer. One set of corrected proofs should be returned promptly to the editor after review. Changes other than the correction of printing errors will be charged to the author at cost.

An order form for the purchase of reprints will accompany proofs. Reprints are ordered directly from the printer.

## Common errors of presentation

The following errors of presentation or interpretation are so common (particularly in taxonomic manuscripts) that they are worth pointing out here. Attention to these points will increase a manuscript's chances of acceptance:

Do not confuse raw similarity (observational, objective) with phylogenetic relationship (inferential, interpretive). The term "affinity" should not be used when "similarity" or "phylogenetic relationship" is meant. Avoid using "close" as an adjective denoting resemblance.

A two-taxon statement, e.g., "[Taxon A] is closely related to [Taxon B]", is meaningless. The minimal form of such statement with any significance is [Taxon A] and [Taxon B] are more closely related to each other than either is to [Taxon C].

Do not refer to taxa as "advanced" (or "apomorphic") or "primitive" (or "plesiomorphic"). Apomorphy and plesiomorphy are attributes of character-states, not of taxa (which are generally mosaics of apomorphies and plesiomorphies; cf. de Queiroz, K. 1988, *Philosophy of Science* 55:238–259; O'Hara, R. J. 1992, *Biology & Philosophy* 7:135–160).

The formula "*Xus* (= *Yus*)" to designate synonymy is ambiguous; it is not clear whether the writer accepts *Xus* or *Yus* as the valid name. In the former case, write "*Xus* (synonym: *Yus*)" and in the latter write "*Xus* (correctly known as *Yus*)"; or avoid the construction altogether.

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